Volume

1

SAMPLING AND ANALYSIS IN THE ARCTIC MARINE BENTHIC ENVIRONMENT

VOLUME 1
REVIEW OF METHODS

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SAMPLING AND ANALYSIS IN THE ARCTIC MARINE BENTHIC ENVIRONMENT

VOLUME I

REVIEW OF METHODS

The Environmental Protection Service wishes to encourage user comments and suggestions on the content and utility of this review. Any comments concerning the content of this report should be directed to:

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Additional copies of this report and the companion report (Volume 2; Guide to Practice) can be obtained from the above address at a cost of $25.00 per report.

PREFACE

This report is the first part of a guide for managers and planners involved in the collection and analysis of environmental data from marine sediments in arctic regions. This report provides an overview of available methods and serves as a background document to Volume 2, a guide to planning and selection of suitable procedures.

The report is organized into three sections: (1) an introduction outlining the background and rationale for the project; (2) a review of sampling methods; and (3) a review of analytical techniques. **Because of the large volume of information available, especially for analytical techniques, the review is not exhaustive. Rather, the review has focussed on methods in current use as well as relatively new methods which appear to offer significant improvements.**

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SECTION I: INTRODUCTION

1. BACKGROUND

Marine sediments are a complex mixture of water and solid components of primarily detrital, authigenic and biogenic origins. The detrital component consists of weathered rock fragments from the continents. The authigenic fraction comprises precipitates formed *in situ* either in the water column or in the sediment. The biogenic portion as its name suggests has a biological origin and comprises the skeletal remains of organisms or organic material produced by the degradation of organic tissues (Calvert, 1976). In addition, sediments support a diverse and in some instances rich community of organisms.

Marine sediments are the final repository of most particulate and dissolved contaminants discharged to the sea or nearshore drainage basins. Dilution and dispersion processes in sediments are much slower than those in the water column with the result that sediment inputs tend to be long-term and cumulative. Benthic organisms are exposed to contaminants transported to the sediment and may accumulate the contaminants or their by-products in their tissues. Sediments and the organisms they support can therefore serve as indicators of both short-term and chronic contaminant inputs. Because of their value as monitors, the study of marine sediments and sediment biota is an important area of environmental science.

There is an increasing requirement for environmental information on arctic marine sediments because of offshore hydrocarbon exploration and associated proposed transport mechanisms. At the same time, there is an increasing awareness that methods that have been used for collection, analysis and data reporting need improvement if the data are to have long term value (Thomas *et al.*, 1982). Many of the shortcomings of past studies have been a result of a lack of awareness of the basic requirements of a sound environmental method and the limitations imposed by arctic conditions. Studies of chemical and biological sediment characteristics involve a number of steps from collection through to analysis. As in any data gathering sequence, the end result is only as valid as the weakest link in the chain of operations. In the Arctic, extreme and prolonged cold for much of the year, continuous ice cover over most regions for 8-12 months and the remoteness of the region from major population centres are all major obstacles to sample collection and analysis. As a result, sampling and field techniques have often been the weakest link in arctic marine environmental studies.

Government agencies and industries requested to provide environmental marine sediment data in support of development or to satisfy regulatory requirements are faced with a wide array of potential sampling and analytical methods from which to choose. Choice of methods is made difficult by the lack of consensus as to the best procedures and the requirements of individual situations.

As a step towards coordinating and standardizing data gathering procedures, the Environmental Protection Service (N.W.T.) contracted Arctic Laboratories Limited to prepare a guide for the selection of suitable sampling and analytical methods for collecting marine sediment environmental and benthic organism data in arctic regions. In our proposed approach to this study, Arctic Laboratories Limited suggested that the objectives be carried out in three phases. The first phase, identified the present regulations governing the disposal of wastes and the collection of environmental sediment data in the Arctic and on that basis and after discussions with government agencies, outlined the environmental and related parameters to be reviewed. The second phase which is the subject of this report is a general review of current sampling and analytical methods for parameters identified in Phase I, relating these where possible to the special features and conditions encountered in the Arctic. The final phase is a guide to the selection of suitable procedures for a variety of situations and goals (Volume 2: Guide to Practice). A list of the parameters included in this review is given in Table I-1.

1.1 Elements of an Environmental Method

The general elements of an environmental method as outlined by the ACS Committee on Environmental Improvement (Keith et al., 1983) are:

1) definition of goals;
2) preparation of a plan for sampling, analysis and reporting data;
3) sampling, subsampling and transport;
4) analysis;
5) methods of quality assurance;
6) reporting and documentation.

TABLE I-1

ENVIRONMENTAL PARAMETERS REVIEWED IN THIS STUDY

- Benthic biota (zoobenthos and macroalgae)
- Benthic biota contaminant body burdens - (metals, PAH and synthetic organics)
- Sediment Chemistry

a)	organics	- hydrocarbons derived from petroleum - chlorinated hydrocarbons - phenols and chlorophenols - TOC, oil and grease, COD
b)	metals	- major constituents (Fe, Al, Si, Mn) - trace metals (Hg, Cd, Cu, Pb, Zn, Cr, As Ni, V, Be)

- Metals in Sediment Pore Water
- Sediment Grain Size
- Sedimentation Rates via Pb-210 dating
- Collection and Maintenance of Organisms for Bioassay Testing

The goal needs to be clearly defined at the outset. This involves identifying the problem(s) and the information requirements and the use for which the data are intended. This could range from collection of preliminary information in an area where none exists to the more rigorous requirements of permit compliance or litigation. The goal needs to be clearly defined as ambiguity or too much generalization will make the goal impossible to achieve. Once the goal(s) has been established, a plan should be devised. The plan will define the population to be examined (area, extent, type of sediment, depth, species); the tests to be performed, desired performance characteristics (precision, accuracy, confidence limits); the methods for sampling, measurement and reporting given the performance requirements and intended use of the data; and finally, cost and time requirements. Definition of goals and planning are steps that must be undertaken by management or by a regulatory agency to satisfy the individual needs of different situations. The sequence of events in formulating a plan will undoubtedly be an iterative process. Goals usually require modification when faced with practical limitations. The plan must be modified if the chosen methods are not practical in terms of time, funding, logistics and facilities. After many iterations, it may become obvious that the goal cannot be achieved forcing either a modification in expectations or an increase in the allocation of resources.

1.2 Objectives

The goal of this review is to examine methods for the collection and analysis of the biological and chemical parameters in arctic marine sediments outlined in the Table I-1. The review is not intended to detail individual methods, but rather to point out the relative merits of methods presently in use. Specifically, the review includes the following topics:

1) sampling and analysis of benthic organisms;

2) analysis of benthic organisms - contaminant body burdens;

3) sampling and analysis for contaminant levels in sediment and sediment pore water;

4) means of collecting organisms to be used in testing the toxicity of sediments, drilling fluids and mine tailings;

5) special features of sampling and analysis required to detect the presence of drilling fluids and mine tailings in marine sediments.

Included in the review is an examination of the methods that would be used to implement a sampling and analysis plan once the goal and plan have been established (items 3-6, Section 1.1), including an identification of advantages and shortcomings of the methods. In general, no single method of sampling and analysis exists for any given parameter or situation. Choice of method invariably involves some form of compromise. The following sections provide background information to aid in the planning process. Specific recommendations are outlined in Volume 2 (Guide to Practice).

1.3 Literature Cited

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SECTION II: SAMPLING METHODS

1. INTRODUCTION

1.1 Background

Sampling is just one of many steps in the overall method. However, it is the step in which the investigator has the least control. In the Arctic, sampling is generally the limiting step in the data gathering sequence. Weather and accessibility prohibit sampling at certain times and locations, and remoteness is synonymous with high cost. In addition, bottom sediments in arctic regions are poorly defined so that in most locations, there is no background information available to aid in formulating a sampling plan.

The major steps involved in sampling sediments or biota are: (Kratochvil and Taylor, 1981):

1) identification of the population from which the sample(s) is to be obtained;
2) selection and removal of a valid gross sample from the population; and
3) reduction of each gross sample to a laboratory sample size.

Methods that can be used to carry out these steps are the basis for the formulation of a sampling plan. Preparation of a detailed plan is an essential requirement for a successful program. Effective planning requires a clear outline of the objectives, definition of the sediment or biota to be studied and some estimation or prior knowledge of the natural variability (Eberhardt *et al.*, 1976). Before a plan is devised, a model needs to be developed which should identify all the assumptions that are made about the sediment or biota and the distribution and characteristics of substances to be measured (Taylor, 1981). Sampling objectives can be either descriptive or analytical depending on whether the information is simply to be used to describe the characteristics of sediment or biota of a region or to make comparisons and to form or test hypotheses about various parameters (Cochran, 1977; Eberhardt *et al.*, 1976). The sampling plan should address the following questions:

- where should samples be taken?
- how should samples be collected?

- when to sample for biota?
- what surface area of sediment is required for biota?
- what depth of sediment is required?
- which tests are to be made and how much sample is needed?
- what performance characteristics and methods for quality control are required?

In addition, the plan must address the practical aspects of sampling, including:

- type of sampler
- time of year
- storage containers required
- logistic support needed (shelter, field gear)
- transportation
- positioning
- safety requirements
- choice of personnel

The sampling plan must allow for contingencies based on actual conditions encountered in the field. Equipment malfunctions, poor weather, ice conditions logistic problems and sediment composition can all destroy the best formulated plan. The field investigator may be forced to abandon the whole attempt or, in an effort to salvage something, may make a number of sampling decisions which may or may not satisfy the goal. Consequently a "minimum" program should always be specified and the goals clearly established so that changes can be made that will, as much as possible, be in harmony with the stated objectives. In the end, the success of a sampling program may depend as much on the quality of field personnel and their ability to adapt to unforseen circumstances as to the thoroughness of the original plan.

1.2 Climatic and Ice Conditions in the Canadian Arctic

The effects of weather, ice and restricted daylight present obstacles to any arctic sampling program. These factors will dictate or influence many important aspects of sampling including: when to sample, time required, transportation, equipment performance, clothing and shelter requirements. In preparing a sampling plan, it is essential, therefore, to review the available information for the region of interest regarding expected weather, usual ice conditions and transport restrictions (see also Section II-9).

There are several overviews of climatic and ice conditions which can be consulted including the Pilot of Arctic Canada, volume 1 (Canadian Hydrographic Service, 1968)); volumes 3A and 3B of the Beaufort Sea E.I.S. (Dome Petroleum et al., 1983), and texts such as Bryson and Hare (1974) and Hare and Thomas (1974). Detailed weather observations for stations around the Arctic can be obtained from most weather offices of the Atmospheric Environment Service, Environment Canada.

The marine areas of northern Canada have a distinctly arctic climate. Winter (average temperature below 0°C) dominates the year lasting for 8-10 months. The extreme winter temperatures in this region are not that different from much of southern and central Canada. The major difference is the duration and intensity of cold weather, with mean temperatures often remaining below -30 to -40°C for extended periods. Summers are short and cool with the mean temperature of the warmest month (July) usually less than 6°C in coastal areas.

Although precipitation is generally light across the Arctic (less than 25 cm in most regions), there can be extensive periods of limited visibility due to fog and low cloud in the open water season and blowing snow after freeze-up. Precipitation is greatest in the south eastern Arctic (southern, eastern Baffin Island), while blowing snow is most severe in the central Arctic and archipelago.

The development and break-up of ice is variable year to year and between regions. This factor, as much as weather conditions, will influence sampling plans. Average maximum thickness for first year ice is generally 1.5 - 2 m, with the thickest ice forming in the central Arctic and the thinnest in the eastern regions. (See figure II-1.1).

As a result of these factors, there are two periods during the year when sediment sampling will either be very difficult or impossible. These include: 1) freeze-up when new ice is forming, but before the ice cover becomes stable enough to support sampling operations (1-2 months) and, 2) during break-up in the spring or early summer when ice is unstable and shifting rapidly and before it is possible to operate small vessels. In addition, from late November through to early February, there is limited (or no) daylight. As a result, aircraft operations necessary for field support are restricted and sampling logistics are much more difficult.

An optimum period for sampling in most regions, in terms of weather, ice conditions and daylight, is usually from late March through May. Temperatures are

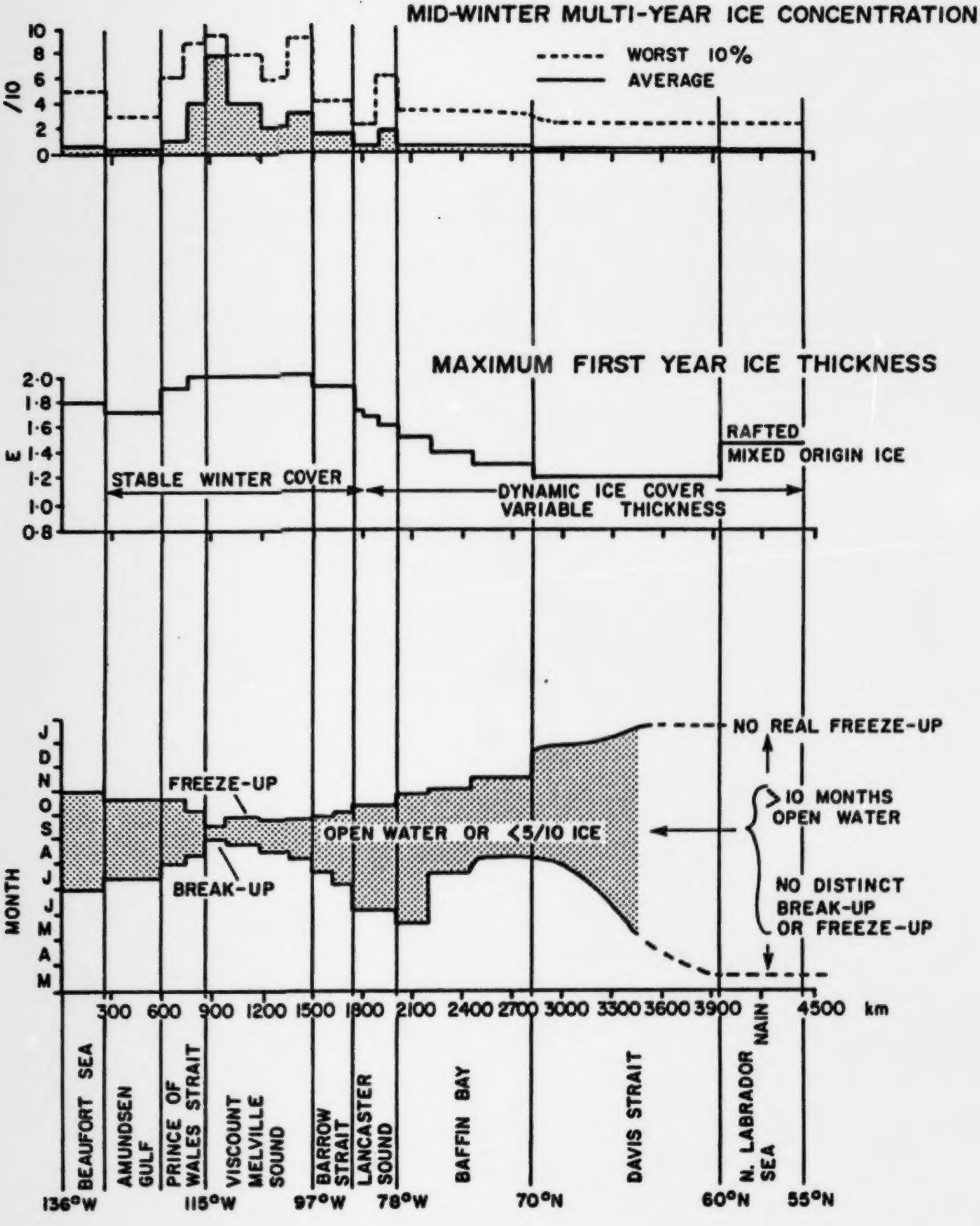

Figure II-1.1. Variation in ice conditions from the Beaufort Sea through the Northwest Passage to Davis Strait (from Dome Petroleum Limited et al., 1983).

moderating, there is 15 h or more of daylight and clear, settled weather often prevails for long periods. In summer, despite warmer temperatures, there is a higher frequency of low cloud and fog in most coastal areas because of open water, with the result that access to the sampling area by air may be difficult. Open water sampling is usually best performed from mid-August to mid-September, although some regions will not clear of ice for a whole season.

1.3 Sampling Theory

> "The purpose of sampling theory is to make sampling more efficient. It attempts to develop methods of sample selection and of estimation that provide, at the lowest possible cost, estimates that are precise enough for the purpose" (Cochran, 1977).

From a statistical standpoint, the sampling of marine sediments and biota shares much in common with other populations. It is impossible to examine all the sediment or organisms in a given area, or all depth horizons. Investigators, therefore, attempt to take samples that will be representative of the whole population. Representative implies that a sample(s) exhibits average properties of the population. However, average properties are not known beforehand, and there is no method to verify that a sample is indeed representative without collecting a prohibitively large number of samples. "Representative" samples, therefore, are the ideal. In practice, something less is achieved unless a sample is defined as representative in a permit or guideline.[1] This, however, will be purely an operational definition. The investigator must make choices and assumptions based on the chemistry or biology of the parameters of interest in defining the target sediment or biota and means to sample it. Sampling theory together with experience and common sense can provide a framework to meet the objectives more efficiently once this has been carried out. A detailed discussion of the general aspects of sampling theory as applied to sample surveys can be found in the text by Cochran (1977). Geological and biological applications of sampling theory are given in the texts by Krumbein and Graybill (1965) and Green (1979) and in several recent review articles (Kratochvil and Taylor, 1981; Eberhardt et al., 1976).

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[1] There is an important distinction betwen a "sample" and a "sub-sample". A single sample of sediment or a single organism will rarely, if ever, be representative of the sediment or biota as a whole. A sub-sample however, is usually considered to be a representative split or portion of a sample.

Several assumptions and decisions must be made to delineate the "target population". This will be a function of the overall goal. Sediment characteristics vary with location, bottom topography and sediment depth. Body burdens of contaminants in benthos vary with tissue type and undergo seasonal cycles. The goal of the program may be (1) descriptive, testing simply for the presence of a substance(s), surface variations or depth dependence within the sediment in a given geographical area, or (2) analytical and involve testing hypotheses or changes (monitoring). The study area can be related to natural boundaries such as depth contours, shorelines, or bottom topography. Often, however, arbitrary boundaries must be chosen, based on distance and direction from a reference point. Once an area or portion of the sea bottom has been defined, the depth of sediment, type of organism, the type of tissue, the sediment horizon, sediment size fraction or component (pore water) has to be specified. If recent inputs are to be measured sampling should be restricted either to the upper centimetre or to the depth of expected mixing due to bioturbation. If the historical level or "norm" is required, then analysis of deeper sediment horizons or "reference" sites will be required. Usually, sedimentation rates are not known, so conservative estimates must be made. Finally, contaminants scavenged from the water column will be concentrated within the fine-sized portions of the sediment so that sampling the fine-grained sediments is important (Hakanson, 1984). Once the target population has been identified in terms of species, size fraction, depth and area, field logistics and methods of sampling and analysis selected, there are a number of approaches to defining how samples and sites are selected and how many samples should be taken. These are outlined briefly below.

There are basically two statistically defined means of selecting field samples. Samples can be taken at random or in some sort of systematic manner. Samples collected either randomly or in a systematic way can be analysed individually or a composite sample can be prepared.

In general, the less that is known about an area, the more random sampling. However, as sediment characteristics of a region are more fully understood, systematic sampling is a more efficient means of sample selection (Saila et al., 1978).

In all instances, the target sediment must be divided into sub-units. The size and number of sub-units will be a subjective decision. For instance, EPS has recommended that sampling density in support of ODCA applications be a minimum of 1 sample/1000 m^3 (J. Karau, personal communication). Positioning capabilities will also be important as this will dictate the resolution that can be expected.

1.3.1 Random Samples

Random sampling is a method for selecting samples such that each part of the population has an equal chance of being selected. This does not imply a haphazard selection. On the contrary, random sampling is a difficult procedure that requires a great deal of care and planning. The target sediment is divided into a number of imaginary units. This may involve a grid overlay, with grid dimensions a function of detail required and positioning limitations. The units to be sampled are selected randomly. This can be done with the aid of tables. Detailed instructions can be found in Cochran, (1977). The advantage to this type of selection process is that by removing bias, generalizations based on mathematical probability can be made. The most common approach to sampling in an unknown area is usually via some evenly spaced pattern. Although this is simpler and would appear to be valid, this method is more subject to bias, and hence not recommended. Stratified random sampling is another approach to sample selection whereby the target population is first divided into sub-populations which are then treated separately. Stratified random sampling would be a suitable approach, for instance, if a sampling plan required samples from two or more different depth horizons, different sediment types or different species. The sub-populations are called strata, and if samples from each are drawn randomly, the process is referred to as stratified random sampling.

1.3.2 Systematic Samples

As its name implies, this method of sample selection involves a systematic selection process. If the target population is divided into n sub-units, selection of the first unit determines the remaining selection. This type of sampling is often carried out to test a hypothesis when a source of contamination is known or identified, or when sufficient information is available concerning contaminant distributions to allow a more focussed sampling approach. Examples include measuring a depth horizon in a core at specified depth intervals from the surface, and sampling surface sediments in a radial fashion, or in a line at predetermined distances from an assumed source. The results of samples collected in a systematic way may also be tested statistically (Cochran, 1977).

1.3.3 Composite Samples

A practice often carried out is to pool a number of samples, homogenize them, and analyse subsamples of the composite (Piotrowicz et al., 1981). This approach is used where an average compositional value is needed. It can have the advantage in substantial cost saving as fewer analyses will be required. However, though the average of a properly prepared composite and that of a number of individual analyses will be the same, the composite effectively conceals the between-sample variability. This is an extremely important statistic when dealing with sediments which are by nature heterogeneous. The use of composite samples has, therefore, been discouraged (Youden and Steiner, 1975; Kratochvil and Taylor, 1981; Eberhardt et al., 1976).

1.3.4 Selection of Sample Size and Number of Samples

The sample size requirements will be dictated by the requirements of the analytical techniques (Section III), the sensitivity required, the expected variance and degree of segregation and the number of replicates to be analysed. A means of calculating the minimum weight of sample required for a given variance has been developed by Gy (cited in Ingamells, 1974). Gy's sampling constant is defined as:

$$Ms_r^2 / d^3$$

where M is the weight of sample required to attain a relative sampling variance s_r^2 and d is the linear dimension of the largest particles. This relationship shows that a much larger mass of coarse grained material will be required to attain a given level of sampling variance. Visman's "General Sampling Theory" (cited in Ingamells, 1974) can also be used to assess the degree of sediment inhomogeneity and analyte segregation. Procedurally, two series of samples are collected and analyzed with a different weight (one large, one small). The results are used to calculate a homogeneity constant and analyte segregation constant which can be used to calculate the variance of N samples of a given weight:

$$s^2 = A/W + B/N$$

where A and B are the homogeneity and segregation constants respectively and W is the total weight of all samples.

The number of sites to be sampled within the study region and the number of replicates required at each site will depend on the objectives, detail required as well as practical constraints. In descriptive sampling surveys, the number of sites selected and the number of replicates taken will usually be a subjective decision by the investigator based on practical constraints. Regardless of objectives, a compromise must be reached between the number of sampling sites and number of replicates per site based on assumptions about the magnitude of regional variability and the sampling uncertainty.

The number of field samples will be the sum of number of sites, replicate samples per site and the number of subsamples from a single grab or core. If the sampling plan calls for a certain level of confidence in the measurement or involves comparisons or testing specific hypotheses, then it is important that enough sites are sampled, and that enough replicate samples at each site and sufficient subsamples are collected to meet this requirement. The number of samples required to achieve a given level of statistical significance can be calculated if the mean and variance of the parameter in the sediment or biota are known or can be estimated; if the degree of segregation is known; if the distribution of the parameter is known (i.e., Gaussian, log normal, binomal or Poisson); and if the variance due to sampling is known. In most instances, this information will not be available. In these cases estimates would have to be made on the basis of experience and expected conditions. Kratochvil and Taylor (1981), Hakanson (1984) and Piotrowicz et al. (1981) have outlined some statistical means of calculating the number of samples for a number of different situations and assumed analyte distributions. In most cases the actual number of samples will have to be reconciled with cost and time constraints.

1.4 Sources of Error

There are numerous potential sources of error, both random and systematic in sample collection and storage. It is useful to be aware of as many as possible so that steps can be taken to avoid them and to aid in subsequent data interpretation. Some common ones are listed below.

1) Positioning (Section II-2.6): In most instances, especially in deep water, assumed spacing of samples may be in error by 100's of metres. This may lead to both random and systematic errors in subsequent data interpretation.

2) Sampling Devices (Section II-2.1): Samplers will disturb the sediment to various degrees depending on design and use. Use of some samplers results in complete loss of surface fines, as well as sediment compaction. Most samplers do not work well in gravel or coarse sand or when there is a mixed gravel sediment. These sediment types will therefore be under-sampled. Samples may introduce contamination either from materials (positive contamination) or by mixing sediment layers.

3) Sub-Sampling: Usually, only a portion of grab or core sample is stored for analyses. This can lead to a bias if sub-sampling is not carried out in a consistent manner. (See for example, Walton, (1978), for suggested methods).

4) Storage: Storage containers may contaminate the sample while storage conditions may alter sample composition.

1.5 Means of Quality Control in Sampling

Procedures need to be incorporated into the sampling plan to provide quality control and to assess the magnitude of the error in the sampling and sub-sampling procedures. The topic of quality control in the laboratory is discussed in Section III-1.2. The goal of quality control measures are to control sources of error and assess the sampling and storage variability. It is not possible to measure the accuracy of sampling as the "true" sediment composition is not known. Instead, measures of precision obtained by examining distributions generated if a procedure is applied repeatedly to the same population are used. Some elements of quality assurance applicable to sampling are listed below.

1) **Replicate Samples:** At randomly selected sites, more than one sample is collected (includes variability due to sampling equipment, subsampling procedures and substrate heterogeneity).

2) **Split Samples:** From a single collected sample, two or more subsamples are taken and stored (includes variability due to subsampling procedures and heterogeneity within a single collected sample).

3) **Collection of two series of sub samples, each of a different weight:** The results of analyses from each series of weights can be used to calculate Visman Sampling constants for homogeneity and analyte segregation and the expected subsampling variance (Ingamells, 1974).

4) **Field Control Samples:** Uncontaminated sediments are spiked with known amounts of the analyte(s) of interest.

5) **Preservative Reagent Blanks:** Blank levels of reagents used to preserve samples in the field need to be checked.

6) **Control Site Selection:** When an objective of the program is identification of elevated levels of a particular analyte (contamination), a site or sites are usually selected that are thought to be uncontaminated for comparative purposes. Proper selection of control sites is important if data are to be correctly interpreted (sediment characteristics need to be closely matched).

7) **Selection of Proper Samplers:** Samplers must be selected which can retrieve the desired type of sediment, are non-contaminating and can collect a minimally disturbed sample (see Section II-2).

8) **Selection of proper storage containers and storage conditions.**

Field control samples and replicate sample results must be treated with some caution. In field control samples, the added analyte may behave and be extracted differently than naturally occurring analyte. Quantitative recovery of the field spike does not mean naturally occurring analyte will be quantitatively recovered or behave in the same way. Under recovery however, will indicate a problem. Results for replicate samples will include variability due to the sampler, sample handling, storage, as well as the heterogeneity of the substrate. In some areas, the latter variability may be much greater than all other factors. Only replicate samples taken from a known homogeneous sediment can be used to estimate sampler and sampling technique uncertainty (precision).

1.6 Strategies for Industrial Inputs

Offshore hydrocarbon development and mining activities are currently the two main industrial activities producing wastes which are discharged directly to Canadian arctic marine areas. Drilling fluids and drill cuttings from offshore hydrocarbon exploration, primarily in the Beaufort Sea, are the major waste discharged; ore concentrates, entering coastal areas near marine loading terminals at Nanisivik N.W.T. and Little Cornwallis Island, N.W.T., have a smaller influence in terms of total amount of material discharged and the number and frequency of point sources. The sampling and analysis of marine sediment (or biota) to detect the presence of either drilling fluids or ore concentrates requires a knowledge of the chemical characteristics of the material, size distribution of the particulates, volumes of material discharged or lost and the location of the sources. A sampling survey to detect an industrial input is an analytical exercise as comparisons and hypotheses testing would be required.

Drilling fluids are a solution of dissolved materials and suspended solids in fresh water, sea water or oil. The properties and compositon of drilling fluids are variable depending on the carrier liquid, type of well, rock formation and depth (Thomas *et al.*, 1983) Characteristics of different drilling fluids based on carrier fluid are given in Appendix A. Much of the literature on tracing drilling fluid disposal can be found in the *Proceedings of a Symposium on Environmental Effects of Drilling Fluids* (American Petroleum Institute, 1980). Thomas *et al.* (1983) reviewed much of this information and developed a simple model for drilling fluid dispersal.

Suitable tracers of drilling fluids in marine sediments will depend in part on the carrier fluid. Water-base muds and cuttings are routinely discharged to the sea. Oil-base muds are, at the present time, not routinely used in offshore drilling operations in the Canadian Arctic. However, guidelines for their use are presently being developed.

Some potential tracers of drilling muds/cuttings are listed below:

Water-Base Muds (see Appendix A)	**Oil-Base Muds** (see Appendix A)
barium	barium
chromium (when chrome lignosulphonates are used)	synthetic organics (Biocides)
synthetic organics (biocides)	aromatics and aliphatic hydrocarbons associated with base oils

Barium is probably the single most useful tracer because (1) it is abundant in drilling fluids; (2) it usually behaves conservatively in marine sediments; and (3) there are no other significant anthropogenic inputs of Ba to arctic marine sediments.

Ore concentrate from existing coastal mining operations in the Arctic, at Nanisivik and Little Cornwallis Island, can be distinguished on the basis of metal content and particle size. Concentrates are primarily lead and zinc; the most abundant metallic impurity is cadmium (Thomas et al., 1984a)

Thomas et al. (1984b) pointed out that strategies for detecting industrial wastes in marine environments should focus on the sediments for the following reasons:

(a) The sediments are an accumulating medium through two mechanisms:
  1) sedimentation of solid particulates; and
  2) demobilization of dissolved compounds which adsorb onto biogenic and non-biogenic fractions.

  Consequently, the sediments can integrate contaminant effects.

(b) Concentrations of contaminants tend to be higher in sediment than in the water column; this allows the use of higher detection limits (less costly) and leads to a greater confidence in results, and easier interpretation of data than is usually the case for water column effects.

(c) Variability in concentrations of contaminants in sediments on short time and distance scales tends to be small (compared to concentrations in water); it is therefore much easier to obtain "synoptic" data and demonstrate a gradient during sampling.

(d) Samples are easily replicated.

(e) Through the use of sediment cores, historical data on the input of contaminants can be obtained for use as a reference without the need for pre-development baseline studies.

Specifically, strategies for detecting waste drilling fluids and cuttings and ore concentrates in marine sediments will involve the following:

1. **mass balance calculations and selection of suitable tracers; consideration of receiving environment concentrations and oceanographic conditions including dispersal mechanisms.**

The first step is the mass balance calculation. This involves predicting accumulation rates of contaminants in the sediments by taking into account the rate of waste discharge, physical characteristics of the waste and the concentration of significant contaminants in the discharge. The importance of this step should not be underestimated. It will provide a first approximation of the scale of the disturbance and define the detection limits required to resolve the contaminant inputs against natural (background) levels. Another benefit of having an approximate indication of the scale of disturbance is that this information can influence the frequency of sampling and the distance between sampling points.

The natural (background) concentrations of those elements or compounds which are also tracers of the waste discharges will influence the monitoring strategy by setting detection limits. As the natural concentration of a constituent decreases in the receiving environment, it becomes progressively easier to resolve the constituent associated with the discharge source from the background, assuming that detection limits are sufficiently low.

Finally, oceanographic conditions (water depth, water column density structure, currents, tides, water column particulate load, frequency and duration of high energy events, and bedform movements) indicate the likely direction of waste migration, and the rate of dispersion in both the water column and sediments. Such information will help define the requirements for sampling equipment, sample replication, sampling frequency, and station locations (offshore drilling rigs routinely collect site specific oceanographic observations).

2. **modelling the dispersion of the drilling fluids or concentrate (assumed means of dispersion, oceanographic features) to provide a framework for predicting sediment distributions.**

Saila et al. (1978) found for instance that dispersion of heavy metals from a dredge spoil dump site followed a log-normal distribution. Thomas et al. (1983) predicted an approximate log-normal dispersion of metals associated with drilling fluid disposal from offshore platforms in the Beaufort

Sea. The same study predicted a bimodal distribution in Scotian Shelf sediments as a result of the increased tidal influence in dispersion.

3. **using a systematic sampling scheme to choose sampling sites with spacing based on the assumed dispersion model.** For a point source, some sort of radial plan centred on the source is probably most appropriate (Saila et al., 1978; Crippen et al., 1980).

4. **sampling and subsampling must be designed to recover the top cm of sediment undistrubed as this will contain recent input (see Section II-2).**

5. **if the wastes have a well defined particle size range, the sediment should be fractionated according to size and only the appropriate size range analysed to improve sensitivity (Thomas et al., 1984a).**

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2. REMOTE SAMPLE COLLECTION FOR CHEMICAL ANALYSIS

2.1 Introduction

Sediment samples can be collected either remotely from a vessel or through the ice, or directly by means of divers or submersibles. Remote sampling is the most common means of collecting sediment and, in most instances, the only practical one. However, direct collection using divers or submersibles, though more costly and difficult, offers distinct advantages.

Remote sample collection is done "blind". The investigator can not see the bottom, the operation of the sampler, the exact location at which the sampler strikes, or its orientation. Direct sampling allows all these things to be done so that sample collection is more efficient and the integrity of the sample easier to assess. Direct sediment collection methods are, therefore, closer to the ideal of obtaining and retrieving an undisturbed sample to the required depth from the target sediment. Direct sampling is reviewed in Section II-4. Remote sampling methods for chemical analysis are reviewed below.

There are three basic devices which can be used to collect sediment samples from a surface platform: 1) dredges which scoop surface sediment as they are dragged along the bottom; 2) grabs which take a "bite" of surface sediment; and 3) corers which collect a column of sediment. There are numerous individual examples of each of these basic types and there are several reviews documenting their features and operational characteristics (Plumb, 1981; Sly, 1969; Holme and McIntyre, 1971; and Bouma, 1969).

Dredge samplers are of use primarily as a qualitative tool in the study of benthos and their use is reviewed in Section II-3. They are not of use for quantitative analysis and are not suitable for collecting sediment for chemical analyses. Grabs are the main tool for quantitative sampling of benthos (Section II-3). A general outline of available grab and core samplers and their applicability for collecting sediment for chemical analysis is presented here.

The ideal remote sampling device for chemical analyses is one that can obtain and retrieve an undisturbed sample in sufficient quantity for all analytical requirements. The sampler should be easy to use, not contaminate the sample, have a reasonable cost and be effective for use over a wide range of sediment types.

No one sampler satisfies all these requirements; sampler selection, therefore, involves a number of compromises based on the overall goals of the program, the type of sediment and whether or not depth profiles are required. The term "undisturbed" when applied to sediment sampling is a relative one. In most instances it implies that the sample has not been compacted, mixed or had loose surface material displaced. There is, however, no absolute measure of disturbance as the original sediment characteristics are not known and all samplers, of necessity, disturb the sediment where the cutting edge or body of the sampler enters the sediment. Significant disturbance may not be visually obvious or apparent from analytical results. Subsequent data may seem plausible but be in error due to sample loss (Baxter et al., 1981). The degree of disturbance that can be tolerated will vary with the intended use of the data. If the goal is to obtain a bulk sediment, mixing of the sediment will be of little consequence. Where depth profiles are needed, however, mixing and compaction will lead to erroneous results. Loss of the surface fines can not be tolerated if the effects of a recent input are required.

Although there have been no definitive studies undertaken, the design features of samplers that limit sample disturbance are:

a) speed on entry: for the same sampler the degree of disturbance decreases with speed of entry.

b) surface area: the larger the open vented surface area relative to the wall thickness, the less disturbance.

c) cutting mechanism: the cleaner and faster the cutting action, the less disturbance due to mixing.

d) sampler wall thickness: thinner walls give a "cleaner" cut.

Positive contamination of sediments from samplers can arise from introduction of analyte or interfering analyte from the sampler itself, from the operation or lowering wire and from mixing of sediment from different depth horizons. The number of materials available for sampler use is limited since samplers have to be fairly robust. Almost all sampler components are metallic. As in the case of surface disturbance, there are no studies that might indicate how important sampler materials are or how severe contamination effects might be. Steps to limit sampler contamination are based on common sense and caution. Obvious features like flaking metal or paint, rust or corrosion, oil or grease, must be avoided. As a precaution, sediment for metal or organic analyses are always taken away from the sampler body. Plastic liners and core barrels are available for gravity corers and although preferable for metals, are a possible source of contamination for some hydrocarbons. Preferred materials for hydrocarbons are stainless steel and anodized aluminum. It is possible to have samplers Teflon coated, but there are no data to indicate whether this is a necessary precaution.

Sample handling is an often overlooked potential source of error. There is not the same sense of caution that is usually applied to a relatively "clean" process like water sampling. However, care must be taken that clothing, tools, lines, winches and hands do not become sources of contamination.

Beside factors which influence sample integrity, there are a number of other sampler features that are important from a user's standpoint. Obvious items are bulk and weight which will dictate lifting capacity required, ship requirements and ice hole size. More subjective factors are ease of operation and reliability. These latter aspects of sampler design are often crucial to the success of a sampling programme, especially in adverse weather conditions.

Except for very light grabs in shallow water, most remote samplers require a winch to retrieve the sampler and sediment. Lifting capacity and power requirements are important considerations. Approximate lifting requirements for various types of samplers are shown in Table II-2.1. Also, as water becomes deeper it may be more difficult to tell when the sampler is on the bottom and a pinger may be required so that excess line is not played out which may subsequently tangle on the sampler (U.S. Naval Oceanographic Office, 1968).

Grabs and corers are different in design, and the two types of devices have very different applications. Grabs are necessary where a relatively large area (or volume) of sediment is required (i.e., for benthic sampling), and limited depth penetration is acceptable. Corers are required when depth profiles of more than about 15-20 cm are needed. Either type of sampler can be used to sample the upper 15 cm of unconsolidated soft sediments when limited quantities of sediment are required for analysis. Choice of sampler will depend on the objectives, convenience and operational characteristics. Most grabs are limited by the depth of water in which they can be effectively used as their high surface area/weight ratio and shape can cause planing on descent (Bouma, 1969).

All remote sampling devices were designed for use from a vessel. Some designs require a large lifting capacity and are very bulky. Many of these devices are, therefore, not practical for through-the-ice sampling where size becomes a limiting factor.

TABLE II-2.1
LIFTING REQUIREMENTS FOR VARIOUS TYPES OF REMOTE SAMPLERS
(from Moore and Heath, 1978)

SAMPLER TYPE	REQUIRED LIFTING CAPACITY (kg)	TYPICAL CROSS-SECTION (cm^2)
Grab samplers	110 - 250	< 600
	200 - 300	1000
Gravity Corers:		
Open- barrel	200 - 2500	20 - 100
Phleger	70 - 100	10
Multiple (3 - 5 barrels)	250 - 600	10 - 80
Free-fall	0	10-30
Piston	200 - 2500	20 - 50
Box	350-400	400 - 600 (can be as much as 2000)

2.2 Grab Samplers

Grab samplers have a long history. The first samplers were developed almost 100 years ago and most original designs are still in use. All grabs operate by taking a "bite" of the surface sediment. They differ principally in the shape of the jaws, tripping mechanism and mode of entry into the sediment. A summary of commonly used grabs is given in Table II-2.2 and various designs illustrated in Figures II-2.1 and II-2.2. Of the available samplers, there are four (with various modifications) that can be recommended for use in environmental sampling: 1) Ponar, 2) Van Veen, 3) Birge-Ekman, and 4) Smith-McIntyre. The advantages of present versions of these samplers are:

1) large vented top (screen) or opening in the back of the buckets which greatly reduces shock wave induced surface sediment disturbance;

2) hinged tops which close on retrieval preventing washout but provide access to the surface sediment so that the sediment does not have to be dumped out for subsampling; and

3) a relatively straightforward non-disruptive method of penetration and jaw cut.

The effect of shock wave was investigated in a comparative study of a Smith-McIntyre and Van Veen sampler by Wigley (1967). Both samplers had an open surface area of 1000 cm^2. Wigley used recovery of ping pong balls on the sediment surface as observed by motion pictures as a measure of the severity of the sampler produced shock wave. The Smith-McIntyre was more effective than the Van Veen (which failed to collect as many balls). This was directly related to the area of the vented openings in the buckets, as the two samplers, in terms of bucket dimensions, were very similar. (The Van Veen had a much more restricted flow of water through the tops of the jaws). The performance of the Van Veen grab can be improved by "making" larger openings (e.g., Kahl Scientific Bulletin WAP 6172/2; Figure II-2.1) and by incorporating a frame to improve performance on sloped bottoms and to slow entry (Meek and Ray, 1980). Some grab designs do not, however, allow that type of modification. Included in these is the Shipek sampler which has excellent operational characteristics in terms of bite and stability. The Shipek (Figure II-2.2) would be

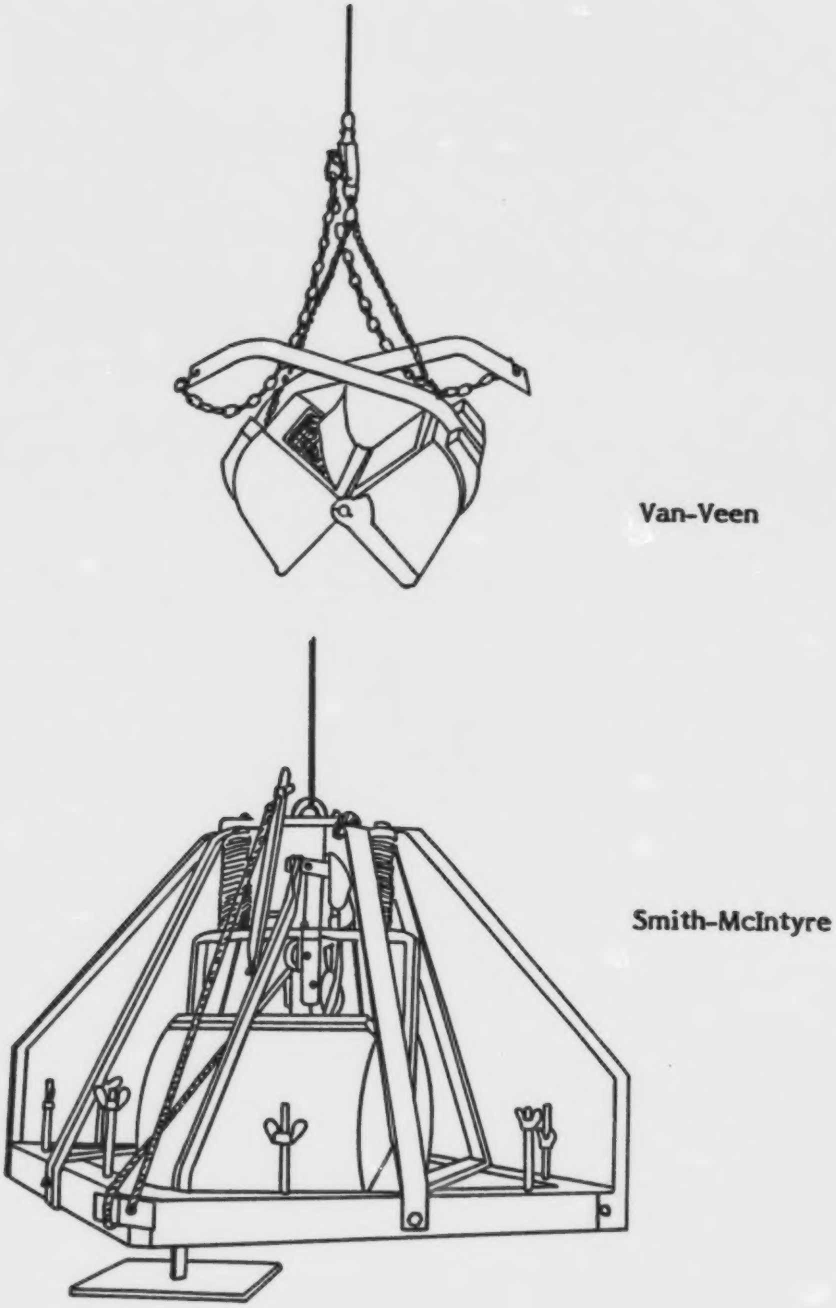
Van-Veen
Smith-McIntyre

Figure II-2.1. A modified Smith-McIntyre grab and an improved version of the Van Veen (from Kahl Scientific).

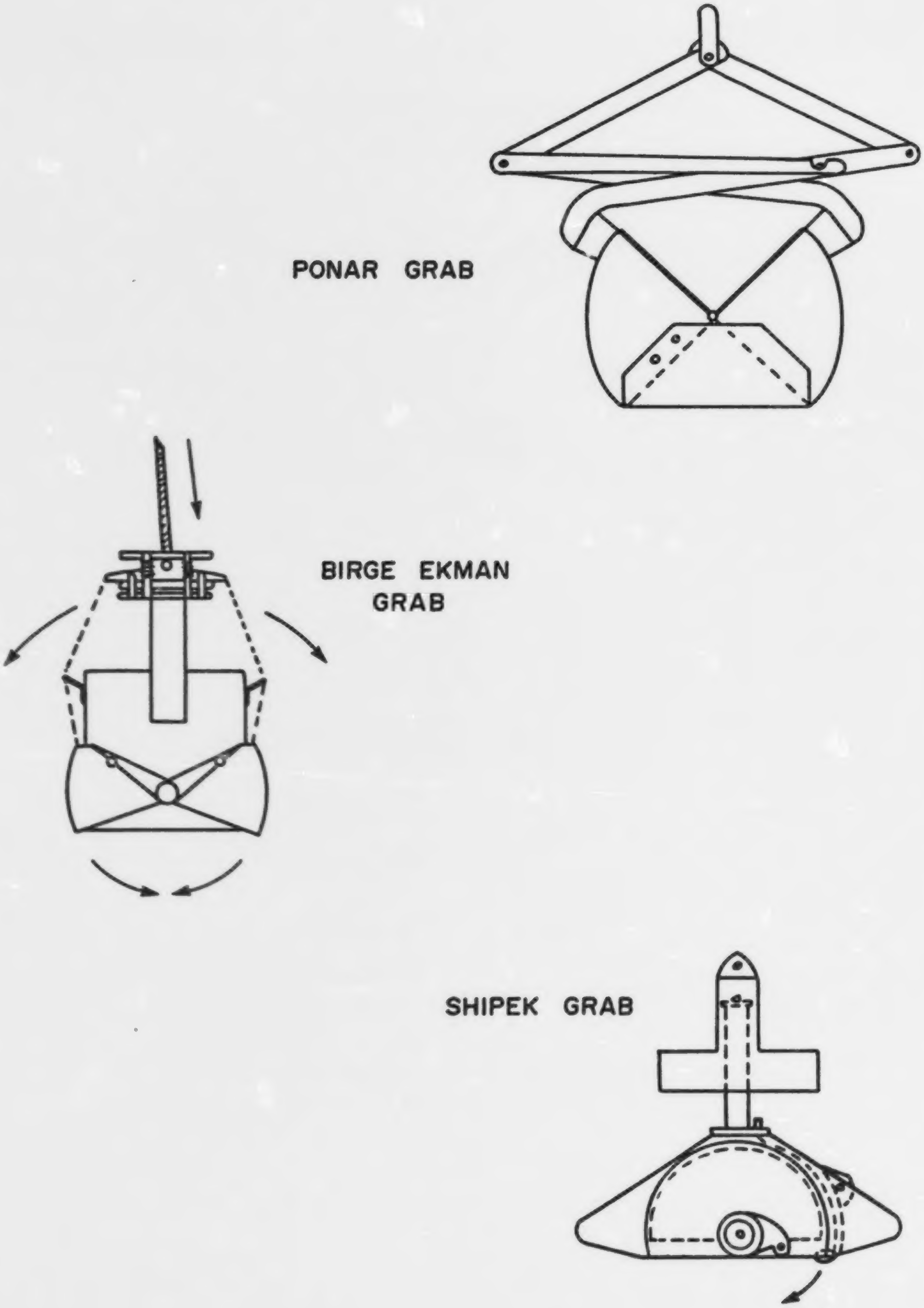
PONAR GRAB
BIRGE EKMAN GRAB
SHIPEK GRAB

Figure II-2.2. Three common grab samplers (from Mawhinney and Bisutte, 1981).

TABLE II-2.2

FEATURES OF COMMON GRAB SAMPLERS

TYPE	WEIGHT (kg)	AREA SAMPLED (cm^2)	MAX. DEPTH OF PENETRATION (cm)	SURFACE DISTURBANCE (relative)	PERFORMANCE IN VARIOUS SEDIMENT TYPES				SUSCEPTABILITY TO SEDIMENT WASHOUT	COMMENTS	REFERENCES
					SOFT CLAY, SILT	SAND	GRAVEL PRESENT	HARD CLAY			
Petersen	34 - 45 (Extra Weights 67)	600 - 2000	20	high (can be reduced with vents in bucket tops)	good	good	sometimes	sometimes	low, except in coarse sediment or if shells, debris present which will prevent jaws closing completely	samples only suitable for bulk analysis (less surface fines); sample must be emptied before subsampling	Plumb, 1981 Sly, 1969 Curtis, 1972 Mawhinney and Bisutti, 1981
Van Veen	19 - 41	- 2000	20 - 30	low-medium depending on size of screen openings	good	good	sometimes	sometimes	low, as above	modified Petersen grab with long scissors-like arms which improve efficiency and penetration; short arms have been used for through-ice sampling	Mawhinney and Bisutti, 1981 Wigley, 1967 Petersen, 1977
Modified Van Veen (Soutar-Van Veen)	- 80	1000	20 - 30	low					as above	mounted in a frame: sampler slides slowly down guides after frame hits the bottom; very stable; minimal disturbance; has been deployed by a helicopter in support of studies close to an offshore drilling rig	Meek and Ray, 1980
Poner	23 - 30	400 - 550	20	low	good	good	sometimes	sometimes	low, overlapped jaws and side plates: washout may occur when gravel prevents complete closure	good all-around sampler: suitable for most sediment types: jaw shape unlike van Veen, Petersen exactly follows arc of cut minimising sample displacement	Plumb, 1981 Mawhinney and Bisutti, 1981 Howmiller, 1971 Hudson, 1970
Smith-McIntyre	45 - 90	1000	20	low	good	good	sometimes	sometimes	low, as for van Veen	basically a van Veen sampler mounted on a frame. Has trigger plates in frame so that open grab is pushed into sediment with springs (force > 35 kg); very stable because of frame; able to sample on slopes; could be deployed by a helicopter as for Soutar-Van Veen	Smith-McIntyre, 1954 Kahl Scientific
Birge-Ekman	5 - 10	250 - 500	10 - 20	low	good	sometimes	no	no	low, as for Petersen	poor stability in all but calm conditions, soft sediment ideal for through-ice sampling because of its compact size. Messenger triggered; spring activated jaws. Jaw shape exactly follows arc of cut minimizing sample displacement	Howmiller, 1971
Shipek	70	400	10 - 20	moderate to high	good	good	good	good	low: has advantages over all other grabs in that bucket closes with separation plane horizontal rather than vertical: washout is minimal even if total closure is prevented.	excellent sampler except for surface disturbance Spring action of bucket gives cleanest cut and widest range of application: sampler is stable but bulky: Bucket is released after retrieval for subsampling	Plumb, 1981 Hydro-Products De Groot and Zschuppe, 1981
Dietz-Lafond Franklin-Andersen Orange-Peel	25 - 30	variable	10 - 20	high					moderate; fines easily lost	not recommended because of excessive shock wave, disturbance of sample and poor operational characteristics; all sample must be emptied out before subsampling	Plumb, 1981 Mawhinney and Bisutti, 1981

useful for sampling in areas of hard substrates (hard clay, gravel), where surface disturbance is less likely and a composite sample is required (De Groot and Zschuppe, 1981).

All grabs, (with the possible exception of the Shipek) do not function well when there are coarse particles in the sediment. Even when sufficient penetration is achieved, large particles caught in the jaws will prevent closure of the jaws and usually result in partial or complete loss of sample.

Of the recommended grab samplers, the Ponar is perhaps the most versatile for collecting samples for chemical analysis. It is relatively compact and yet heavy enough to be effective in a range of sediments. The Smith-McIntyre is the largest and heaviest sampler and will, therefore, be restricted primarily to shipboard use. Its frame imparts good stability however, and it is therefore, effective on a wide variety of bottoms and slopes. A modification of the Van Veen incorporating a frame and slowed entry to minimize surface disturbance has also been produced (Meek and Ray, 1980). The main drawback of the Birge-Ekman sampler is its light weight with the result that it is ineffective on hard bottoms and is more susceptible to planing on descent. It can therefore, only be used effectively in shallow water with weak currents and relatively soft, flat bottoms. Extra weights can be added to improve performance. The Birge-Ekman is the grab sampler of choice for through ice sampling in shallow regions with soft bottoms because of its compact size and excellent operational characteristics in these conditions. The Van Veen and Smith-McIntyre (see Section II-3) are recommended for benthic studies and are therefore samplers of choice when both benthic and chemical sampling are required.

The cost of commercially available grab samplers ranges from \$1,000 to \$6,000. The smaller, simpler models such as the Ponar and Ekman are in the lower price range while the Smith-McIntyre and more elaborate Van Veen modifications will be in the upper price range. A partial list of manufacturers is given in Appendix B.

2.3 Coring Devices

Coring devices of some sort are required whenever depth- related sediment samples are required. Corers can be classified according to the shape of the core and mode of penetration. Most corers have cylindrical core barrels and rely on gravity for sediment penetration. A number of gravity corers have also been developed with square or rectangular shapes (Bouma, 1969). Cylindrical core barrel corers have also been designed to improve sediment penetration by increasing the driving force or by decreasing the resistance to core penetration. These include piston corers, vibra corers, and compressed- air-driven corers. Gravity corers, both cylindrical and box shaped, are the most practical and widely used in environmental sampling. The other corer types are more complex, require specialized equipment and, in some cases, result in considerable core disturbance. Examples of these types of corers can be found in the review by Bouma (1969).

2.3.1 Cylindrical Corers

Because of the internal friction between sediment and core barrel, simple cylindrical gravity corers have limited depth penetration, (10-20 times core barrel diameter depending on sediment type). This will satisfy most applications. However, when deeper penetration is required (in support of an ODCA permit application for instance (Walton, 1978), piston corers can be used. These corers are cylindrical gravity corers with a tight-fitting piston inside the core barrel. Ideally, the piston should remain in the same position relative to the sediment surface creating a negative pressure above the sediment as the core barrel penetrates. The negative pressure counteracts the frictional forces between the barrel and sediment allowing increased penetration compared to an open core barrel. Cores up to 30 or 40 m in length have been taken using this technique in pelagic and lacustrine sediments. The operation of a piston corer is illustrated in Figure II-2.3.

The operation and description of various examples of both gravity and piston corers has been reviewed by Bouma, 1969; Moore and Heath, 1978; Sly, 1969; and U.S. Naval Oceanographic Office, 1968. Common examples of cylindrical gravity corers are compared in Table II-2.3 and a typical corer illustrated in Figure II-2.4.

Several interesting modifications have been developed using a basic gravity corer design. Multiple corers (several core barrels on a single corer) have been developed and are useful for comparative studies, establishing precision of sampling and determining small scale sediment variability (Kemp et al., 1971). Another corer with a specialized use that may be useful in the Arctic is the "free" corer. This device, as its name implies, is deployed free and is returned to the surface by buoyancy. The corers have two parts: 1) a bouyant assembly composed of a strobe light or beacon to facilitate recovery, sufficient bouyancy to lift a core to the surface, release mechanism and recoverable core barrel; and, 2) an expendable weight and casing. Figure II-2.5 illustrates its operational phases. This device allows cores

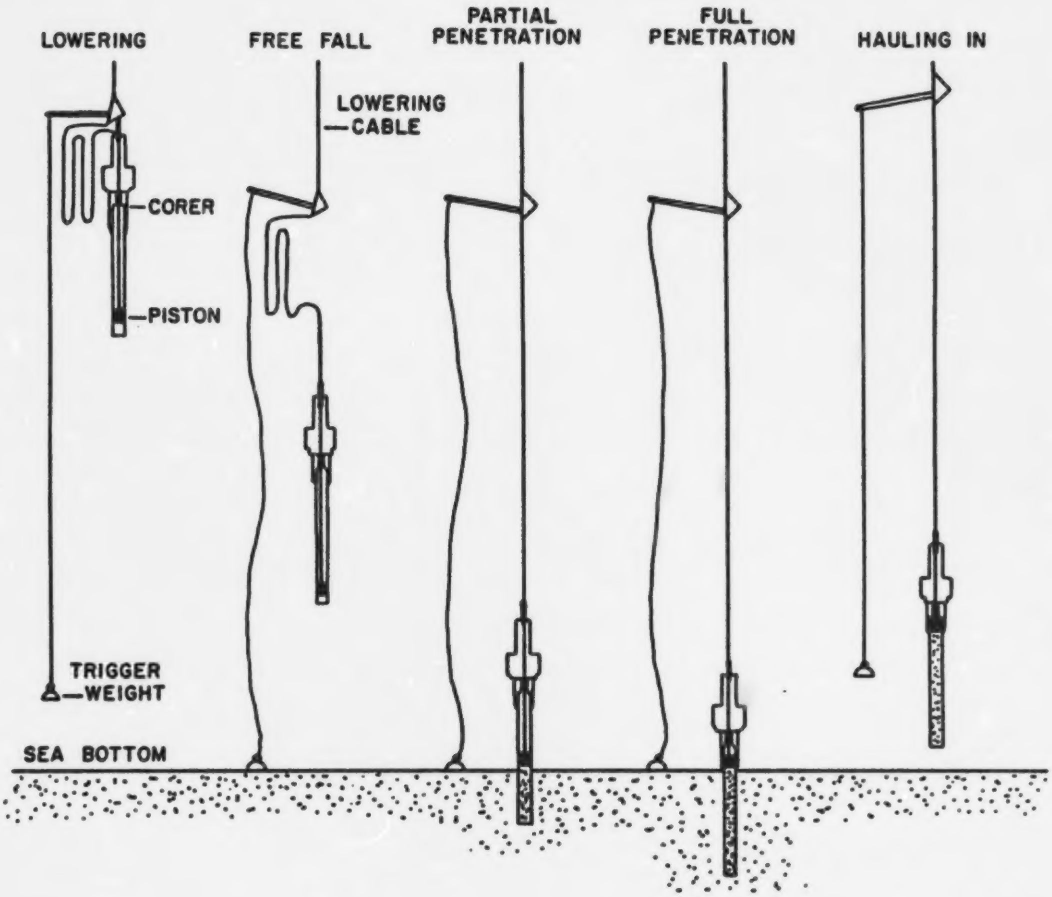
LOWERING
FREE FALL
PARTIAL PENETRATION
FULL PENETRATION
HAULING IN
LOWERING CABLE
CORER
PISTON
TRIGGER WEIGHT
SEA BOTTOM

Figure II-2.3. Operation of a piston-type gravity corer (from U.S. Naval Oceanographic Office, 1968).

TABLE II-2.3

COMMON GRAVITY CORERS

EXAMPLES	APPROXIMATE WEIGHT (kg)	DIMENSIONS	SURFACE DISTURBANCE (relative)	DESIGN FEATURES	SEDIMENT TYPES	COMMENTS	REFERENCES (See Appendix B)
Phleger, Alpine	28-45	variable: core barrels usually small diameter (4 cm)	moderate	very simple design; no moving parts: uses a one-way valve system on top. Water pressure forces this up on descent. Most models have a fixed weight (e.g. Alpine): some have variable weights and stabilizer fins.	soft, sandy, semi-compacted: not a good choice for coarse-grained sediment	commercially available from several suppliers: all are steel construction with metal barrels: plastic liners and core catchers available.	Kahl Scientific Mawhinney & Bisutti, 1981 Bouma, 1969. Sly, 1969
custom-designed Phleger-type:	7 without weight. up to 7 kg per each lead weight	variable:	low-moderate (decreases as diameter of core barrel increases)	Usually aluminum frame, stainless steel cutting head; clear acrylic core barrels: variable lead ring weights; one-way valve consists of rubber bung. No stabilizer fins.	as above	easily constructed	Mawhinney & Bisutti, 1981 Thomas et al. 1983 Sly, 1969
Benthos	up to 70	core barrel 6.6 cm: overall corer diameter ~25 cm	low-moderate	reliable design: spring activated flapper valve; stabilizer fins.	soft, semi-compacted or compacted fine-grained; not a good choice when gravel present or in sand	commercially available	Benthos Corp.
Triple Corer or multiple corer	28 (steel tubes) 21.5 (acrylic)	three (or more) core barrels: dimensions as above	low-moderate	stainless steel pipe or acrylic tubes.	clays, muds, silts	in-house modification	Kemp et al. 1971 Moore and Heath, 1978
Free Corer	85	overall diameter ~25 cm	unkown likely moderate	designed to be dropped from helicopter or vessel; no attachment: consists of i) recoverable core barrel and buoyant core chamber - check valve, and ii) expendable weight and casing	as above	available commercially from Benthos and Hydro-Products: application to arctic open water sampling from helicopter or fixed wing aircraft or when winch not available; expensive.	Bouma, 1969 (Figure II-2.4)
KB^{TM} core	8 or 18 without core barrel	5 cm barrrel: overall diameter: ~15-20	reported to be low: depends on speed of entry	has fixed weight and a messenger-activated valve closure that does not require water pressure to keep it open.	soft sediments	commercially available; stabilizing fin, extra weight available options.	Wildlife Supply Company
Craib (hydraulically damped)	44	5.7 cm diameter core barrel	low	hydraulic damper and ball closing device at bottom for sandy sediment: corer is mounted within a frame (Figure II-2.6).	soft sediments as well as sand	hydraulically damped; cores are 10-15 cm long	Craib, 1965 Baxter et al., 1981

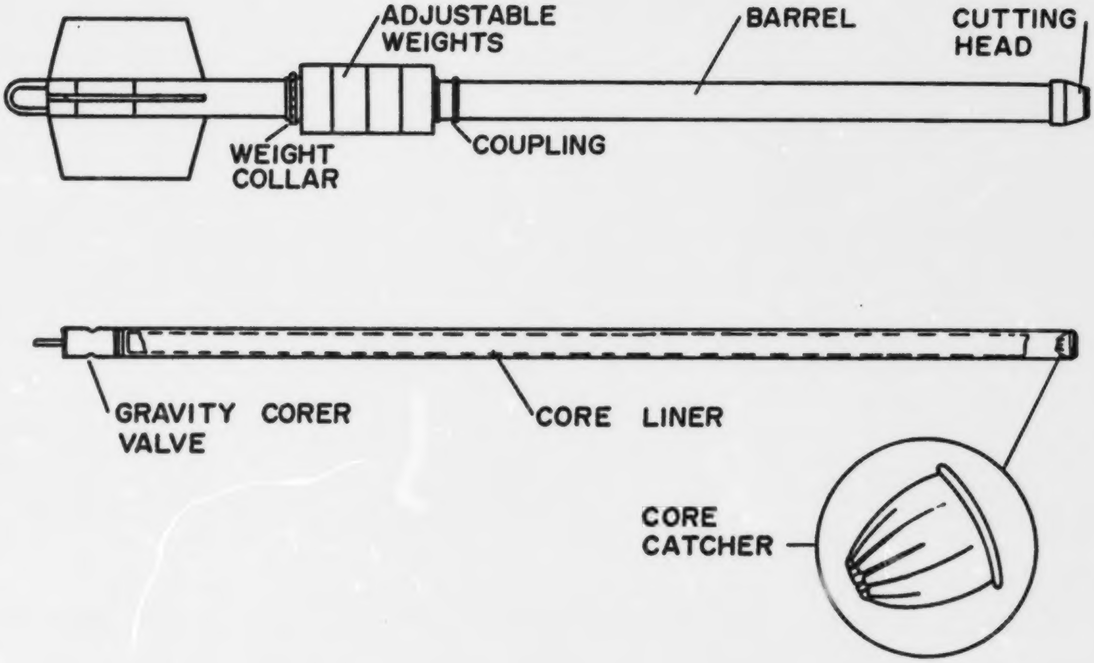
ADJUSTABLE WEIGHTS
BARREL
CUTTING HEAD
WEIGHT COLLAR
COUPLING
GRAVITY CORER VALVE
CORE LINER
CORE CATCHER

Figure II-2.4. A typical gravity corer with core catcher (not to scale; from Walton, 1978).

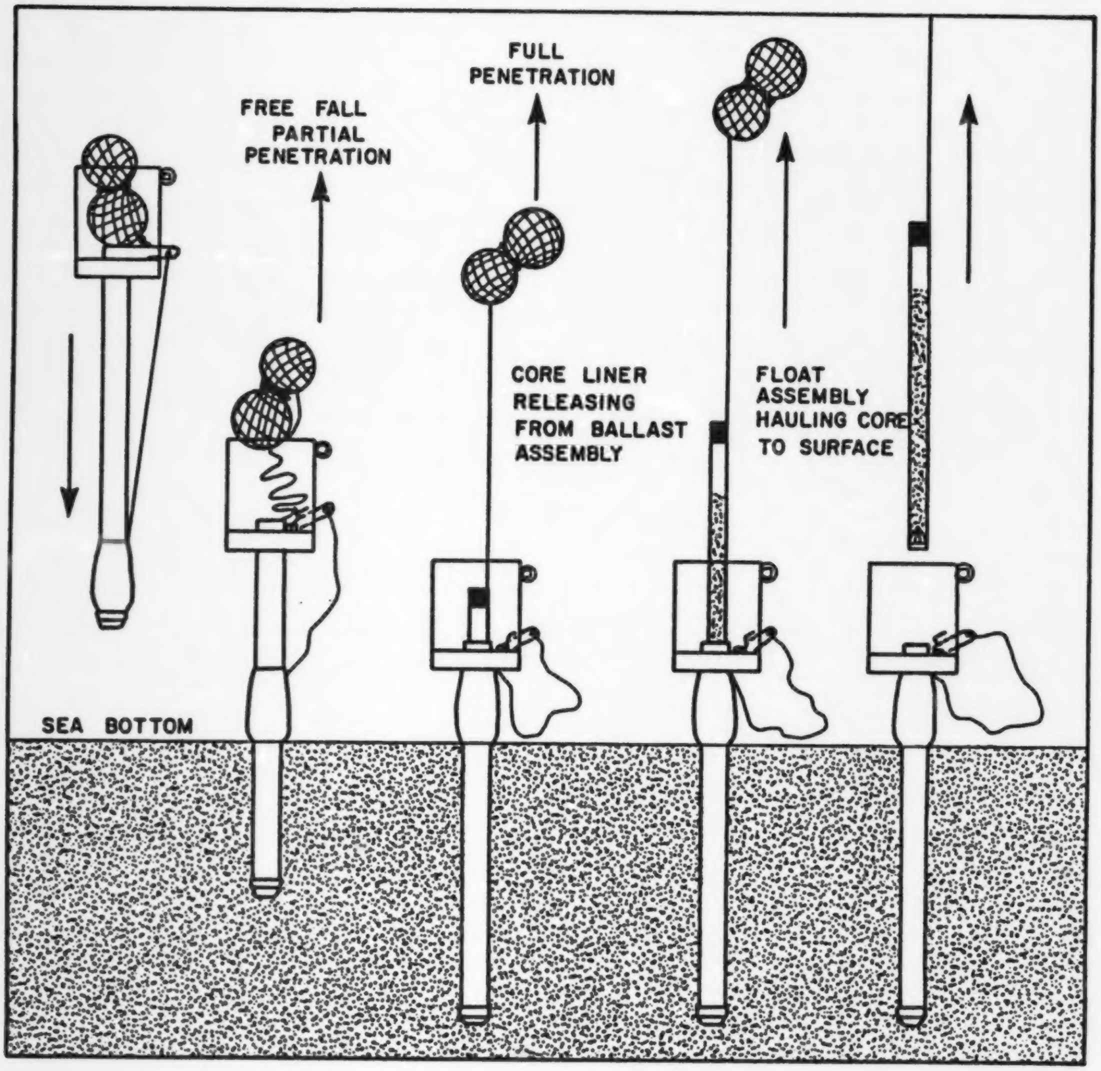
FULL PENETRATION
FREE FALL PARTIAL PENETRATION
CORE LINER RELEASING FROM BALLAST ASSEMBLY
FLOAT ASSEMBLY HAULING CORE TO SURFACE
SEA BOTTOM

Figure II-2.5. Operation of free-fall or boomerang type corer (from U.S. Naval Oceanographic Office, 1968).

to be taken without a winch and could be used in the Arctic from a helicopter or small vessel where only a small number of cores are required. The disadvantages are the lack of flexibility in core barrel dimensions and the expense associated with taking a number of core samples.

Gravity corers do not perform well in coarse sediments or sand. Van de Bussche and Houbolt (1964) developed a cylindrical gravity corer designed specifically for sampling marine sands. The novel feature of the corer is that after the corer is withdrawn from the sediment, it is automatically inverted, preventing loss of sample. The authors reported penetration (and retrieval) of up to 1.5 m of sand. The Craib corer (Craib, 1965), a hydraulically damped gravity corer, has a ball closing device which has been successfully used in sandy sediment. This corer is designed to take very short (15 cm) undisturbed cores (Table II-2.3; Figure II-2.6).

All cylindrical corers tend to disturb the surface sediment because of the shock wave produced during free fall. The degree of disturbance will be a function of the speed of impact and surface area presented. The slower the rate of entry, the less disturbance (but also less penetration). Open core barrels are better than closed ones as these allow water to pass through freely. In this respect, piston corers will create a larger shock wave. Photographic records of a large piston corer deployed to sample pelagic sediments indicated that large portions of the surface sediment (up to 1.5 m) were totally displaced or compacted (McCoy, 1972). After impact, a properly deployed piston corer will cause less compaction of the sediment. However, piston corers will not be suitable for collecting surface sediment. It is common for gravity corers to be used with piston corers, the gravity corer serving as the trip weight for the piston corer release mechanism and for obtaining surface sediment. This is a complicated system and may lead to false samples if deployed from a rolling ship (McCoy, 1972).

Controlling the speed of entry of a gravity corer can be difficult. Burns (1966) found that free fall of 2-3 m was the distance required for all corers to reach terminal velocity. Slowed entry, therefore, requires a winch controlled rate of descent, a tripping mechanism (as in piston coring) that releases the corer to free fall less than 2 m or some sort of damping mechanism. The importance of slowed entry to retention of surface sediment characteristics was illustrated by Baxter et al., (1981). They found that cores obtained with a slow entry corer (Craib, 1965) caused far less disturbance of the sediment than a standard gravity corer allowed to free fall with a similar diameter core barrel. Using Cs-134 as an indicator they found the free fall corer displaced all Cs-134 from the surface, while the slow entry corer collected

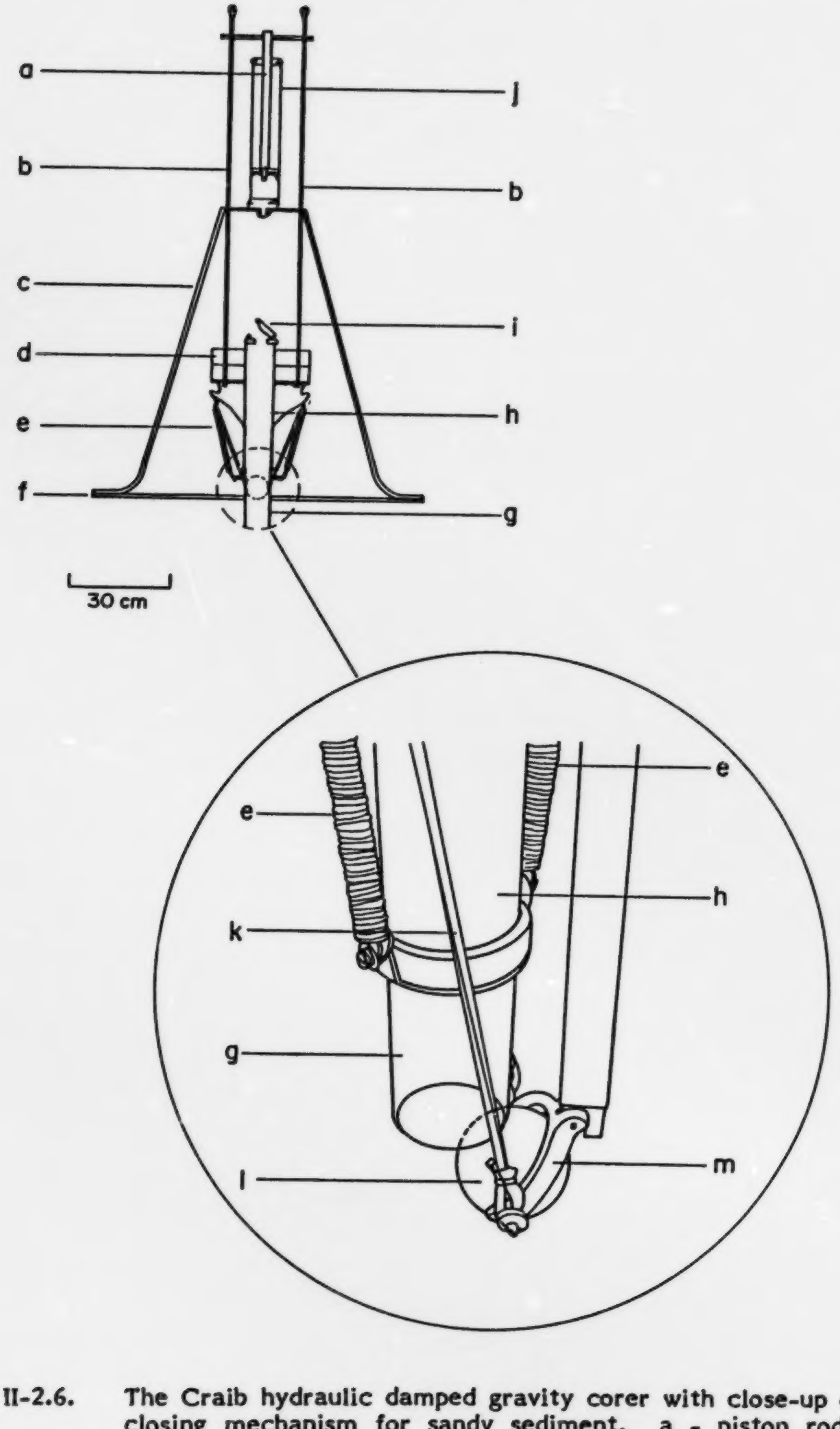

Figure II-2.6. The Craib hydraulic damped gravity corer with close-up of the closing mechanism for sandy sediment. a - piston rod; b - vertical support rods; c - tapered verticle frame; d - lead weights; e - springs; f - horizontal circular frame; g - acrylic core tube; h - tubular housing; i - hinged seal cover; j - hydraulic damper; k - rubber elastic cords; l - rubber ball seal; m - yoke.

relatively undisturbed surface material . There have been no studies to indicate at what speed surface disturbance becomes severe. This will vary with sediment properties, sampler design and the amount of unconsolidated material. It would be prudent, however, to reduce the speed of entry to the lowest level that will still allow sufficient depth penetration whenever recovery of surface fines is required.

In geological or geotechnical applications, it is usual to insert a device into the cutting head of cylindrical gravity or piston corers to prevent loss of the core after pull-out and during retrieval. The different types of core catchers available have been reviewed by Bouma (1969). Ideally, these devices will not disturb the sediment during penetration or restrict the core diameter. The most common type of core catcher is one made of thin copper or brass "fingers" attached to a ring. The fingers point into the core barrel and are bent inward giving them a domed shape. This type of core catcher will almost always distrub the sediment however, especially the surface fines and is not recommended. Other designs involve spring activated valves and are more complex, although they are usually more effective in preventing core loss. All core catching devices present the risk of sediment disturbance or in the case of spring loaded devices, contamination (from spring lubricants) and are not recommended for environmental sample collection.

From the available studies, features of cylindrical corers that are desirable and should be considered in choosing a particular sampler are:

1) mode of penetration: corers that can penetrate to a sufficient depth at reduced entry speed are preferable.

2) diameter of core barrel: larger diameter cores are desirable because of the need for sufficient sediment for subsequent analyses, because of less disturbance of the core as a whole and because contamination from the corer will be less significant. Walton (1978) recommended a minimum of 6 cm I.D.. Wall thickness should be the minimum needed for the expected impact force.

3) weight and lifting requirements: corers with variable weights (i.e., weight can be added or removed) are desirable because the corer weight can be adjusted for a particular sediment or desired depth of penetration.

4) materials: stainless cutting heads are best; clear acrylic core barrels have the advantage that the core can be inspected after retrieval without removing it. Plastic liners are useful features as they reduce contamination for trace metals and speed up corer use.

5) stabilizer fins are useful for deep water or areas with high currents assuring that the corer will strike the bottom in a vertical position.

6) core catchers ensure a higher success rate in the retrieval of cores but can cause considerable disturbance and mixing of the sediment. They should not be used unless a sample cannot be retrieved otherwise. A good seal on the end of the barrel is usually sufficient to "hold" the sediment in the barrel. Valve systems that seal tightly are, therefore, desirable.

The cost of corers varies directly with complexity. The simplest gravity corers can be built or purchased for under $1,000, while a small piston corer may cost from $3-5,000.

2.3.2 Box Corers

Box corers were introduced in the late 1950's. Since that time they have been modified and refined to improve the operational characteristics, (Bouma, 1969; Bruland, 1974). Box corers are all gravity corers with large cross-sectional area (either rectangular or square) and limited depth penetration. Because of the large surface area, these samplers are the best available for collecting undisturbed cores and sampling at the sediment-water interface. The large area also means a larger amount of sediment is available for a given sediment horizon while the shape of the barrel allows a side to be removed facilitating core examination and sub-sampling.

Standard boxes are fabricated from steel or anodized aluminum. Karl (1976) has developed a plastic liner which reduces potential metal contamination and speeds up sampling. Most box corers require a frame for support as the samplers are top-heavy and very unstable. All corers use some sort of shovel seal for the bottom of the box to prevent loss of sediment on retrieval. This feature allows samples to be retrieved even in very loose, unconsolidated sediment and does not affect the integrity of the core. As with grab samples, these shovels may not seal properly because of rocks, debris or shells.

The major drawbacks of box samplers are the limited depth penetration, large size and higher cost (commercial models cost between $5-10,000). The bulk of this type of sampler prevents it being of use in through-ice sampling. Despite this, box corers come closest to the ideal remote sampler for near surface sediments. A typical box corer design is shown in Figure II-2.7 while features of several box corers are compared in Table II-2.4.

2.3.3 Summary

The best type of corer will depend on the goals of the sampling program, depth of water, length of core required, sampling platform (ice or ship), the lifting capability, the types of analyses to be performed on the sediment and the type of sediment.

No gravity corer functions well in very coarse sediments, and if a sample is essential from this sediment type, alternate means will be required (e.g., diver operated corers, vibra corers).

For most environmental data, the upper 50 cm or less is required. For this depth of penetration, there are a number of choices. Table II-2.5 outlines recommended corers for a range of applications. If both surface features and depth related features are important, then two separate samples will be required as all corers which yield long cores either displace or seriously disturb the upper few cm of sediment. A partial list of manufacturers of corers is given in Appendix B.

2.4 Contingency

It is important that the limitations of samplers be considered. No one sampler will perform well at all depths and in all types of sediments. Although samplers are generally robust, equipment breakage or loss can occur. Sampling plans need to include a contingency for different bottom types and for equipment failure (as a result of damage or loss). An allowance for a back-up sampler should be made and the use of more than one type of sampler should be considered in sampling sediments of unknown texture.

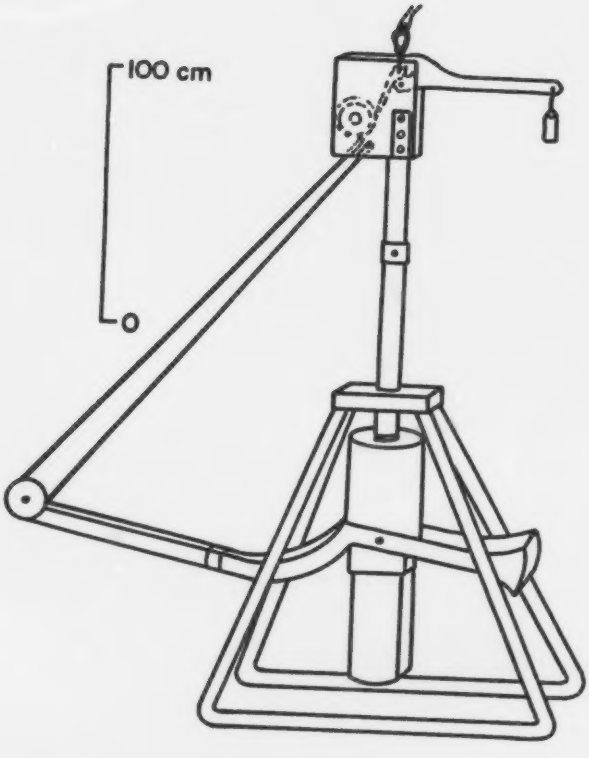

Figure II-2.7. Box corer (from Bouma, 1969). Most designs are similar to the one shown. Bruland (1974) used a box corer with two shovels.

TABLE II-2.4

FEATURES OF BOX CORERS

REFERENCE	DIMENSIONS OF BOX	WEIGHT (kg)	FRAME DIMENSIONS	BOX MATERIAL	OPERATIONAL FEATURES
Bruland, 1974	20 x 20 cm 130 cm high	350 (up to 280 kg of weights can be added)	200 cm x 100 cm	anodized aluminum	uses two overlapping shovels to seal bottom of box: spring loaded plate seal at top of the box shut on retrieval, open on descent
Bouma and Marshall (1964) Bouma, 1969	20 x 30 cm 45 cm high	~ 500 (320 kg of weight)	150 cm x 150 cm	3mm stainless steel	box open at top; single levered shovel seals bottom of box on retrieval
Texas A & M Sampler (cited in Bouma, 1969)	30 x 30 cm 91 cm high	> 600 (filled boxes weigh over 180 kg)	trapezium 120 cm on longest edge	4.7mm stainless steel	box open at top; improved versions of above sampler
Kogler, 1963 (cited in Bouma, 1969)	15 x 15 cm lengths of 1 - 4 m	can use up to 14 weights, 50 kg each	none	steel, PVC coated on interior	one way valve on top of weight stand, core nose has internal spring activated plates to prevent loss of core
Kahl Scientific Bulletin WAB-1464/1	10 x 25 cm 30 cm high	210 (with weights)	120 x 65	stainless steel	box has removeable side wall: box is sealed on top on retrieval with a flexible, thick rubber sheet

TABLE II-2.5

SELECTION OF A SUITABLE GRAVITY CORER

LENGTH OF CORE (cm)	TYPE OF SEDIMENT	SHIPBOARD USE	THROUGH ICE SAMPLING	COMMENTS
50 cm or less (surface)	loose, fine-grained	box corer if sufficient lift capacity or cylindrical gravity corer with wide diameter core barrel (10 cm or more); slow entry; short core barrel (< 100 cm)	as for shipboard sampling except box core not advised because of bulk, difficult to transport, requires larger hole and has too many moving parts	core catchers should not be used; clear PVC or acrylic core barrels preferred for metals; steel or anodized aluminum for organics
	consolidated, fine-grained	as above; except more weight or faster entry speed required	as above	as above
	coarse grained; sand and/or gravel or mixed with large pebbles, rocks	successful coring will be difficult; box core because of shovel seal may perform best; several attempts will likely be required to obtain a core or coring may not be successful at all	as for shipboard sampling	if sample is essential and water is shallow enough; divers should be used
up to 200 cm or 300 cm	loose, fine-grained	cylindrical gravity corer; small diameter barrel (< 10 cm); extra weight and free fall entry > 3 m; small piston corer	as for shipboard sampling; piston corers may not function well in extremely cold weather	separate core required for near surface as this will be compacted/lost; large lifting capacity (up to 1000 kg) required; stabilizer fins useful
	consolidated, fine-grained	as above, but extra weight required	as above	as above; stabilizer fins essential; greater lifting capacity
	coarse grained	generally not possible		
greater than 300 cm	loose, fine-grained	piston corer; large vessel and winch required	piston corer; probably not feasible due to lifting requirements, size of corer; clearance	as above
	compacted, or coarse sediment	not feasible with gravity type corers		

2.5 Sub-Sampling

After retrieval of a core or grab sample, a suitable sub-sample must be taken and stored until analysis can be carried out. Preferable storage containers and conditions are described for each analyte in Section III.

Sub-sampling is usually carried out in the field and thus is a part of the field or sampling program. There are no definitive rules for sub-sampling, only general guidelines which have evolved from the cumulative experience of field personnel and intuitive feelings as to the degree of care or caution required.

General guidelines can be summarized as (Walton, 1978; Plumb, 1981):

1) Immediately after retrieval, core barrels should be stoppered or jaws of grab samplers secured to prevent accidental loss of sample.

2) Sampler must be kept upright.

3) Surface water must be carefully removed (leaving surface fines undisturbed). If the water is extremely murky, surface disturbance will have occurred and fines must be allowed to settle, water filtered or a new core collected. This is perhaps the most critical step except when only bulk sediment properties are required (Walton, 1978).

4) Before sub-sampling, appearance of the core or grab should be noted, along with any obvious features (sediment type, colour, structures, fauna, odour). Any structures such as wood chips, large stones, etc., should be removed or not sampled.

5) For grabs where only bulk (non-depth related) analyses are required, the contents can be dumped onto a board or plastic sheet and sub-sampled according to Walton (1978). For depth related features, a small core can be taken from the interior part of the grab through the hinged tops. Sediment near the grab walls should be avoided.

6) For cylindrical cores, the complete core can either be frozen for sub-sampling in the laboratory or sub-sampled after collection. Some type of piston extruding device will be required for immediate sub-sampling

(Kemp et al., 1971). Frozen cores can be thawed slightly and extruded intact then cut with a saw. Alternatively cores may be first sectioned longitudinally (Walton, 1978; Bouma, 1969), depending on the number and types of analyses.

7) Box cores can either be sub-sampled by removing one side (Bouma, 1969), taking cylindrical corers in the horizontal plane through pre-cut cylindrical holes at set depths in the box wall (Bruland, 1974) or by taking small cores vertically from the surface. Sub-sampling in the horizontal plane is only recommended in very loose, high water content sediments which can be extracted with a syringe.

8) Tools and utensils for sub-sampling must be of a suitable material for the type of analysis to be carried out. For trace metals, plastic scoops or core tubes should be used. For organics, metal (stainless steel, aluminum) materials should be used.

9) As much sample as possible or practical should be stored for analysis. Minimum requirements must be known (Section III).

10) The order in which sub-samples are taken should be consistent with goals and the potential for contamination. Samples for trace constituents (metals, organics) are usually taken first.

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3. REMOTE COLLECTION OF BENTHIC INVERTEBRATES

3.1 Introduction

Sampling of the benthos over a range of bottom types (soft mud to rock) and diverse biotic elements will require several different types of gear and techniques. Each of the sampling and processing techniques has limitations and a variable efficiency for different members of the biota (Table II-3.1).

For macrobenthos, the major limitations of methods arise during sampling. The sparsely distributed and the more mobile members of the macrofauna are difficult to sample quantitatively. If they are active surface dwellers (epifauna) they may dodge or escape the sampler; if they are burrowers (infauna) they may retreat too deeply into burrows to be caught in most grabs. In either case, they are often widely dispersed or "patchy" so that the area sampled may not be representative.

For smaller organisms, which also are often patchily distributed, additional sampling problems tend to arise during treatment and processing where the aim is to separate efficiently animals of a wide range of sizes from one another and from the sediment grains. The divisions of macro (> 0.5 mm), meio (< 0.5 mm) and microbenthos (< 0.1 mm) require different techniques for each group. The primary emphasis in this review is on macrobenthos readily separated by sieving. Sampling of the meiobenthos has been thoroughly treated by McIntyre (1969), Hulings and Gray (1971) and McIntyre and Warwick (1984).

3.1.1 Preliminary Sampling

Decisions affecting the choice of procedures for quantitative macrobenthos surveys are more effectively made after preliminary observations have been made, especially in areas which are poorly known. A small-scale pilot survey should be conducted, possibly including observations from diving, underwater photography and television. Qualitative sampling by dredge or trawl can also provide information on the fauna and flora and features of their distribution.

3.1.2 Arrangement of Stations

Sampling design should be based on the objectives and corresponding hypotheses to be tested in the benthos study (see Section 5, Volume 2). If there are

TABLE II-3.1

SUMMARY OF METHODS OF BENTHOS SAMPLING

TYPE OF EQUIPMENT	TYPE OF INFORMATION	SAMPLING HABITAT	ORGANISMS OR PARAMETERS	TYPICAL SAMPLING AREA	UNIT OF MEASURE	LIMITATIONS OF USE	REFERENCES
1. Dredge	Qualitative or semi-quantitative	Hard or soft substrate	Epibenthos	Unknown	Comparative	Open water, clear of ice	Mohr et al., 1957 Lee, 1973
2. Trawl	Qualitative or quantitative with odometer	Hard or soft substrate	Epifauna; biomass, density	Estimated from trawl odometer	g - m^{-2} no. -m^{-2}	Open water, free of obstructions	Squires, 1962 Carey and Ruff, 1977 Carney and Carey, 1980
3. Sled	Qualitative or quantitative with with 4. and 5.	Hard or soft substrate	Epifauna; biomass density	Estimated from odometer or tow duration	g - m^{-2} no. -m^{-2}	Open water, free of obstructions	Holme and Barrett, 1977
4. Still camera	Qualitative or quantitative with 3.	Hard or soft	Epibenthos; density	Various (e.g.) 0.5 - 1 m^2	no. -m^{-2}	Good visibility, no specimens	Cross and Thomson, 1982 Johnston et al., 1969
5. Television	Qualitative or quantitative with 3.	Hard or soft	Epifauna; density	Various	no. - m^{-2}	Good visibility, no specimens	Holme and Barrett, 1977 Heath and Thomas, 1984a.
6. Grab	Quantitative	Soft or fine sediments	Infauna; biomass, density	0.1 or 0.2 m^2	g - m^{-2} no. -m^{-2}	Soft sediments, limited area	Petersen, 1977 Carey and Ruff, 1977
7. Airlift	Quantitative with quadrat or cylinder	Hard or soft substrates	Epibenthos and infauna; biomass, density	0.05 to 1m^2	g - m^{-2} no. -m^{-2}	Shallow depths, good visibility, diver operated	Barnett and Hardy, 1967 Finnish IBP-PM Group, 1969

no pronounced gradients in environmental conditions or sediment texture, a random allocation of sites within a grid over the study area may be appropriate. Sampling should be random at some level of the sampling design. If there is a significant gradient of conditions such as depth or salinity within the study area, then stratified random sampling may be more appropriate (e.g. Coleman, et. al., 1978). For example, samples may be randomly arranged within relatively homogeneous subareas or strata in numbers which are proportional to the size of the stratum. At each station, replicate samples should be taken to adequately sample the number of species present and to measure sampling variability within and between stations.

To assemble a composite description of the benthos and its environment through use of various samplers or analytical techniques, it is recommended that the different samples be collected as close together as possible. For example, in correlating the benthos and the sediment properties, the sediment sample should come from the same grab sample as the fauna, since two successive hauls can vary considerably in sediment type. When subsamples are removed, the surface area and volume of subsamples should be determined so that the area and volume represented by the benthos sample can be adjusted accordingly. Photographic or video images of the bottom before or during sampling can greatly aid interpretation of benthos-sediment relationships.

3.1.3 Size and Number of Replicates Required

For quantitative macrobenthos sampling, it is generally considered that a sampler collecting an area of at least 0.1 m^2 is required. Criteria for selection of sampling equipment are reviewed in Section II-2 . For a given study area it is important to determine the number of samples at each station necessary to provide valid estimates of the number of species present, the distribution and abundance (population density and biomass per unit area) of the benthos. This can be done by constructing a species/area cumulative curve (Holme, 1953; Jones, 1957; Ursin, 1960, Holme and McIntyre, 1971; 1984). An example is shown in Figure II-3.1. The minimum number of sample replicates is indicated by the position of the asymptote of the recruitment curve. Williams (1964) and Gray (1981) have presented theoretical discussions of the species/area problem and the use of the log-normal distribution in estimating the total number of species present.

Longhurst (1959), however, has presented evidence from a long series of 0.1

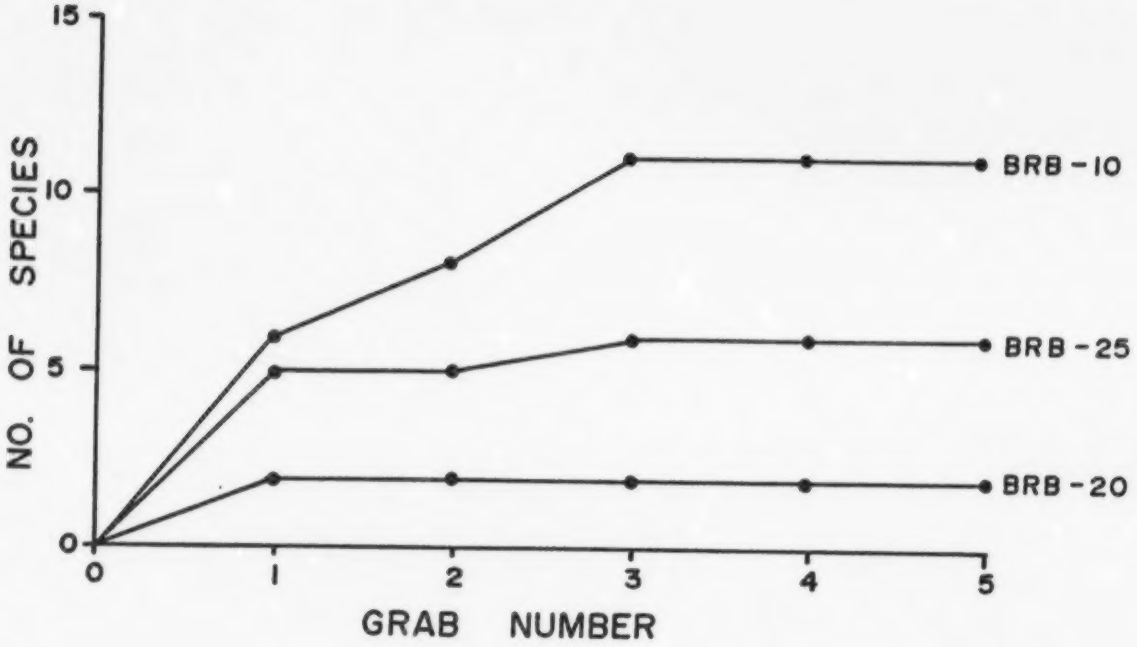

Figure II-3.1 An example of a species/area cumulative curve (taken from Carey, 1980 for three stations between 5-25 m in the Beaufort Sea). Each grab represents an area of 0.1 m^2.

m^2 replicate grab hauls from which he questioned the validity of using cumulative recruitment curves for defining the minimal area for representative macrobenthos sampling. He showed that the abundant infaunal species may be well represented in the first few hauls (i.e., before the asymptote is reached) whereas the sampling of rarer species may not give a valid distribution per unit area even when the asymptote is reached. He considered that a standard five-grab station (covering $0.5 m^2$) is a logical and satisfactory choice, both in terms of ship time and effectiveness of sampling. He found that five hauls provided adequate information on the density and distribution of the abundant species and enumeration of between 50 and 70 percent of the rarer species, which would have been collected in a 20-haul sample. Faunal indices such as total population density and total biomass also appeared to be adequately estimated by five replicate hauls. Carey (1980) stated that five $0.1\ m^2$ grabs in the nearshore area (5-25 m) of the southwestern Beaufort Sea gave unbiased representation of the total molluscan infauna as indicated by asympototic species accumulation curves for all stations sampled. In the examples provided in Figure II-3.1, the minimum number of sample replicates for molluscan infauna is three grabs, but five grabs were taken to show this.

In the course of a preliminary survey for planning of a benthos sampling program, the collection of 10 or more replicate benthos samples at one or more stations would provide the basis for testing the validity of a standard five-haul replicate series. The value of this approach is especially apparent in studies to be conducted in areas in which the fauna and flora are not well known. In the absence of a preliminary study or suitable background information, a series of five replicate samples at each station is advised.

If the principal aim of the benthos survey is to monitor for possible changes in biological parameters, then it is important to consider the following two criteria associated with statistical sensitivity:

1) the probability of rejecting the null hypothesis when it is true (generally referred to as the α probability or Type I error); and

2) the probability of accepting a null hypothesis when it is false (referred to as the β probability, or Type II error)

The complement of β, i.e. $(1-\beta)$, is known as the "power" of a test; it defines the probability of correctly detecting experimental effects, such as differences among sampling stations.

The probabilities of Type I and II errors and minimum detectable differences among sampling stations can be expressed for a given biological parameter. The distribution of sampling resources (i.e. number of stations, degree of replication and sampling frequency) can then be allocated according to project resources (ship time, budget), practicality of the sampling strategy and desired sensitivity of the analyses to be performed. Examples and discussion of this approach have been presented by Scheffe (1959), Winer (1971), Saila et al. (1976) and Gordon et al. (1980).

The number of samples required to achieve a desired precision or range of sampling variance can be estimated from results of preliminary sampling (e.g. Cochran, 1977; Elliott, 1977; and Green, 1979). For example, consider that the mean density of a benthic population is to be estimated within a range of plus or minus 20 percent, with a 5% chance of a Type I error (= 0.05). Preliminary random sampling over the study area has yielded a sampled distribution which is normal, with estimates of the sample mean and standard deviation given by $\bar{x}$ and s, respectively. Confidence limits on the mean are expressed as:

$$\bar{x} = t_{1-(0.5\alpha)}(s / n^{1/2})$$

where **t** is Student's t-statistic and **n** is the sample number to be determined.

The desired range of the mean of plus or minus 20 percent of the mean, therefore, has 0.95 confidence limits given by:

$$0.2\,\bar{x} = t_{.975}(s / n^{1/2})$$

where the only unknowns are the t-statistic and the sample size, n. Although t is a function of its degrees of freedom (n-1), for large sample sizes t is a very weak function of n and $t_{.975}$ is nearly 2. Thus,

$$0.2\,\bar{x} \sim (2)\, s / n^{1/2}$$

or $$n \sim (2\,s / 0.2\,x)^2$$

If the estimated value of n is large, substitution of the corresponding tabulated t-statistic will produce little difference from the above expression. If the estimated **n** is small (<20), then several iterative solutions for **n** and **t** are necesssary. To include some margin of safety, it is advisable to round up to the nearest multiple of 10.

Estimation of sample size under sampling designs other than simple random sampling have been discussed by Elliot (1977), Green (1979) and others.

3.2 Qualitative Sampling

3.2.1 Dredging and Trawling

Exploratory qualitative sampling of the epifauna traditionally has been conducted with a variety of biological dredges and trawls (Gunter, 1957; Holme, 1964; Holme and McIntyre, 1971, 1984). Qualitative sampling of epibenthos by dredges or trawls in the Arctic has been reported by MacGinitie (1955), Mohr et al., (1957), Squires (1968, 1969), Carey and Ruff (1977), Lee (1973) and Wacasey (1975).

The original "naturalist's dredge", which is a modification of the commercial clam or oyster dredge, has been commonly used with only slight changes since the early work of Muller (1779), Forbes (1859), Thomson (1873) and others (Figure II-3.2). A biological dredge is basically a metal frame to which is attached a bag of chain, netting or heavy canvas. The frame, which may be rectangular, triangular or circular, is usually less than about 1.2 m across. The dredge is towed slowly (0.5 to 1 m/sec) on a single warp for 10 to 15 minutes. A weak link (Figure II-3.2.) which breaks first under heavy strain to prevent loss of gear and to improve safety is used in case of mishaps during the tow. Early dredges were used mainly for collecting new species of epifauna by skimming the surface of the seabed. Bucket and conical dredges (Robertson, 1868; Borley, 1923; Powell, 1937) and the deep-cutting dredges of MacGinitie (1948) and Forster (1953) (Figure II-3.3) have been more effective in obtaining deeply burrowing infauna as well as epifauna.

Trawls, such as beam, Agassiz, and otter trawls, also have been used for qualitative sampling of the epifauna (Gunter, 1957). By skimming over the seabed with a large mouth area, these nets are effective for collecting larger or scarcer epifauna and varieties of demersal fish, cephalopods and crustaceans associated with or feeding on the bottom. The **beam trawl** is used commercially for fishing inshore stocks of fish, shrimps and prawns. The net mouth is spread by a beam or bar of about 2 to 4 m length which is raised off the bottom by a pair of skids or runners (Figure II-3.4). The lower leading edge of the net is attached to a weighted chain forming a footrope. The chain stimulates the epifauna to rise off the bottom. However, because the footrope curves behind the headrope at the top of the net which is attached to the beam, mobile epifauna or fish disturbed by the footrope cannot escape upwards (see Figure II-3.4). In addition to animals retained in the

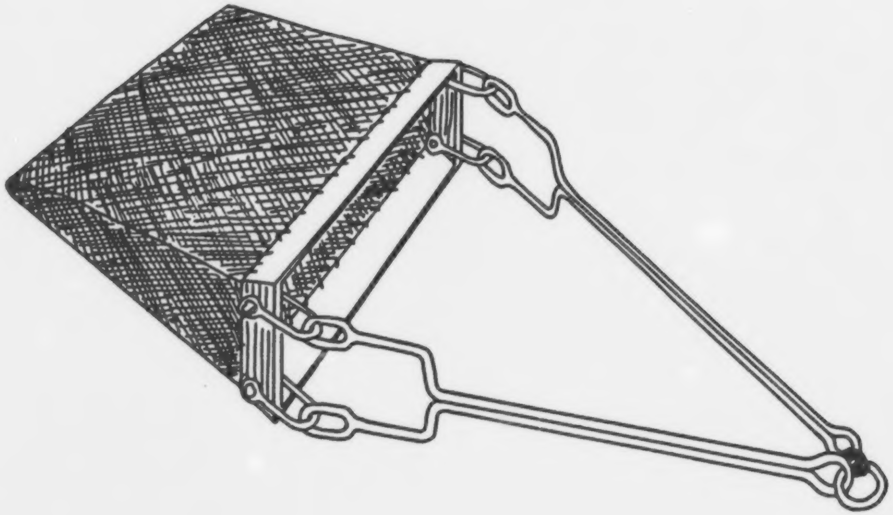

Figure II-3.2. Naturalist's rectangular dredge as supplied by the Marine Biological Laboratory, Plymouth, England. Note that a metal ring is fitted to one towing arm only; the other is connected to the ring by a weak link of hemp rope. (redrawn from Holme and McIntyre, 1971).

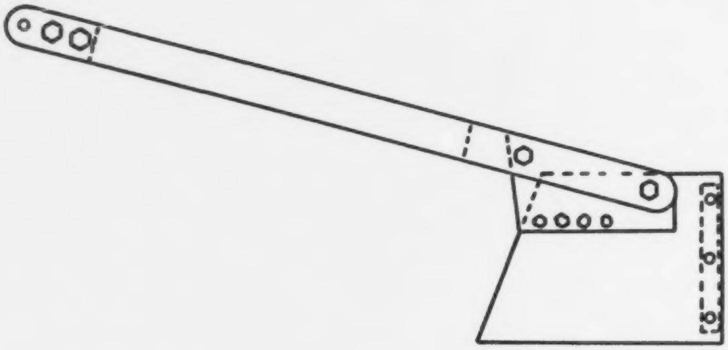

Figure II-3.3. Forster anchor dredge as supplied by the Marine Biological Association, Plymouth, England. (redrawn from Holme and McIntyre, 1971).

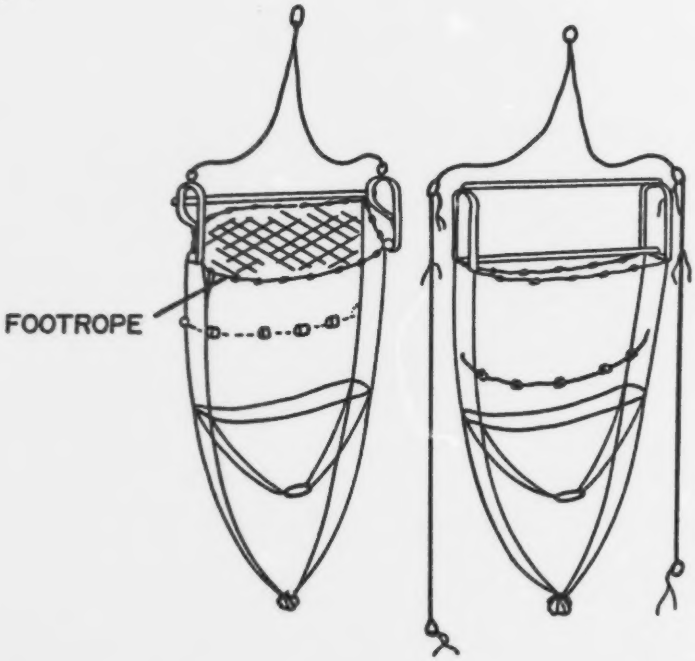

Figure II-3.4. Beam trawl. Left, as used on the "challenger"; right as modified by Agassiz and Sigsbee, the "Agassiz trawl". (redrawn from Gunter, 1957).

codend, many small epifauna may become attached to the fine net meshes. Thus, beam trawl catches can be a significant supplement to collections taken by dredging during epifaunal surveys.

The **Agassiz trawl** (Figure II-3.4) is a modification of the beam trawl designed for fishing equally well upside down or rightside up in deep-sea trawling where it is not possible to control which way the trawl lands on the bottom. Because the head- and foot-ropes are interchangeable in function, they are of the same length. As a result, very few fish are caught by the Agassiz trawl.

The **Otter trawl**, which is the basic net type used in modern commercial bottom trawling, is a large net with a weighted footrope and a headrope equipped with floats. The wings of the net are spread by a pair of otter boards or "doors". The doors are usually separated from the net by ground lines or cables. Details of net configuration and rigging vary from fishery to fishery. Commercial trawls are usually operated by two winches for the warps, and a net drum. They require considerable skill and experience to operate successfully, especially in rough weather. Although designed primarily for catching fish and shrimp, commercial otter trawls also capture larger epifauna in the coarse meshes typically used. Smaller versions of the otter trawl (3-7 m wide) with liners of finer mesh sizes (Steven, 1952; FAO, 1965) have been widely used from small research vessels in ecological studies of fish and epifauna (Mearns and Allen, 1978).

A principal disadvantage of dredging and trawling is that the results are qualitative or at best semi-quantitative (Holme and McIntyre, 1971, 1984) unless a distance metering device is used because the surface area sampled cannot be accurately determined (e.g., Carney and Carey, 1980). It is possible, however, to standardize the speed and duration of tow in order to obtain estimates of population density which are comparable with results obtained under similar conditions. It is widely recognized, though, that most dredges and trawls collect only a fraction of the epibenthos, and the infauna hardly at all; the results, therefore, represent minimum densities (Holme, 1971: Eleftheriou and Holme, 1984). The main advantages of dredging and trawling compared to grab sampling are that the larger, more sparsely distributed epifauna are more likely to be collected; and surface areas of compact or hard substrates where grab sampling is not feasible may be sampled.

Dredging and trawling in arctic waters is limited to the open-water season and to locations free of significant concentrations of ice floes.

3.2.2 Remote Optical Recording

Within the last thirty years, the use of optical recording equipment designed for underwater operation has provided new sources of descriptive information on the benthos and the sea bed. Underwater still and television cameras have been operated directly by divers and submersibles or remotely from ships. Camera equipment has also been attached to towed gear such as trawls or left positioned for extended times on the bottom. Many underwater photographic applications have been highly specialized and have required unique equipment. The development of more standardised underwater optical techniques, however, has resulted in improved availability of photographic and television or video equipment from several manufacturers. This section indicates the range of equipment and some of the applications of remote underwater photography and video techniques to benthos surveys in the Arctic.

Sampling with dredges, trawls or grabs is necessary in benthos work which depends on accurate taxonomic identification, estimation of biomass, chemical or morphological examination of organisms or sediment analyses. Photographs and video recordings, however, can provide valuable supplementary information on sediment conditions, distribution of epifauna and organism-sediment relationships. In areas with rocky or uneven bottoms, optical recording techniques may be one of the best ways to obtain information on benthos. When performed in exploratory surveys optical recordings can also be valuable for planning benthos surveys by indicating the distribution of bottom types or faunal asssemblages to be sampled in the study area.

The principal limitation to most optical recording techniques for benthos surveys is the frequent occurence of high turbidity in shallow coastal waters, especially in estuaries. In the Arctic, poor underwater visibility is usually associated with the seasonal distribution of turbid, low salinity water from major rivers such as the Mackenzie. Most marine (high salinity) areas of the Arctic Ocean, generally have good underwater visibility due to low levels of primary productivity and local run-off. Another limitation for use in the open-water season is that remote camera and video operations are also hampered by rough sea conditions.

3.2.2.1 Remote Still Photography

Remote photography can be used for quantitative estimation of epifauna, especially when combined with other sampling equipment. Some samples of procedures and applications are described below.

In remote photography, it is advantageous to shoot a large number of frames at each lowering, since much of the effort is connected with preparing, launching, lowering and retrieving the equipment. Remote cameras are often triggered by a small weight hung below which trips or switches when touching bottom. An electronic flash synchronized with the camera shutter is fired, a signal is relayed to the surface and the film is advanced to the next frame. Between sites, the equipment can be raised several metres while the vessel proceeds or drifts to the next position. Craig and Priestley (1963) and Owen et al. (1967) have described seabed cameras of this type.

McIntyre (1956) compared benthos estimates from a large study area made by trawl, grab and remotely triggered still camera. Photographs gave the most reliable estimates for the less common epifauna and also clearly indicated the pattern of their distribution over the seabed. Photography also gave better results than the other methods for the more abundant epifuana which were distributed singly or in small or isolated aggregations. In contrast, photographs of the sediment surface are unreliable for estimating abundance of infauna; misleading results can be obtained by trying to estimate density of infauna from surface tracks or burrows (Owen et al., 1967).

Rhoads and Cande (1971) and Germano (1983) have described a sediment-profile camera which permits high-resolution in situ observation and sampling of soft mud bottoms. The optical system of the camera is not limited by high water turbidity because the optical path consists of air and distilled water. It is depth-rated to 2,000m. The sediment-profile camera differs from conventional underwater cameras by its capability to make vertical slices of the sediment-water interface and to photograph the sediment and infauna in profile. High resolution (> 0.06 mm) images of an area of up to 320 cm^2 of sediment profile can be taken with each exposure (Germano, 1983). This REMOTS® (Remote Ecological Monitoring of the Seafloor) camera system is available commercially.

Conventional underwater cameras used together with other sampling equipment can provide valuable supplementary observations. For example, a camera has been attached inside a large bottom sampler, the 0.56 m^2 Campbell grab, to photograph the undisturbed bottom just before the grab strikes (Menzies et al., 1963; Emery et al., 1965). Wigley and Emery (1967), discussing the additional information obtained by this technique, found that grab sampling alone tends to underestimate the fauna at the sediment-water interface.

Cameras may also be attached to the headline of a bottom trawl or the frame of a sled or dredge. Craig and Priestly (1963) and Dyer et al. (1982) have described trawl headline cameras which are suitable for large-scale surveys of epibenthos and demersal fish. These systems have been used to study populations of the epibenthic shrimp, Pandalus borealis, in the Arctic (Blacker, 1971; Kanneworff, 1979). Still photographs taken at intervals during tows make it possible to correlate the catch with the bottom type over wide areas, and to estimate the efficiency of the gear. Laban et al. (1963) and Rice et al. (1982) have used cameras attached to epibenthic sledges to obtain supplementary photographic information in deep water. Holme and Barrett (1977) showed that underwater television and still cameras, combined with a benthic sled can be used for quantitative surveys of the epifauna on the continental shelf.

Remote still photographic equipment is generally custom-built for specific applications; the cost will likely be in the range of $2,000 - 4,000 for an automatic camera and accessories.

3.2.2.2 Remote Underwater Television

Closed-circuit underwater television or video has the additional advantage over conventional photography of continuous viewing at the time of recording and during replay. The ability to record moving subjects is very useful in the study of demersal fish and mobile epifauna and their reactions to sampling gear. If artificial light is needed, however, the effect of the illumination on the behaviour of the subjects must be considered. Like a roving eye, a television camera can quickly survey large areas of sea bed, including sedimentary features and distribution of epifauna.

Commercially available television cameras can be used for underwater surveys by lowering them from an anchored or drifting ship by their power cable (preferably supported by an auxilliary rope), or by mounting them on a frame, sled, submersible, pan and tilt unit or other underwater device (e.g., Holme and Barrett, 1977). They can also be hand-held by divers. Surface control of video and audio recording, focussing, illumination, camera orientation and proximity to the bottom make this technique extremely versatile for continuous recording of epibenthos, benthic habitat and features such as ice scouring or artificial habitat modification. Remote underwater television was used during benthos surveys in the vicinity of marine dredging and artficial island sites in the Beaufort Sea (Heath et al., 1982 a,b;

Heath and Thomas, 1983, 1984 a,b).

Black/white underwater television systems can be purchased for $7,000 - 10,000 or leased in the Arctic for $250 - 300 per day. Colour television systems cost about $17,000 - 20,000 to purchase or $450 - 500 per day to lease.

3.3 Quantitative Sampling

The general aim of quantitative sampling of macrobenthos is to obtain reliable estimates of three indices:

i) the number of species ("species richness");

ii) the number of individuals or population density (total and of each species); and

iii) the biomass (total, and by species).

These measurements are expressed in terms of unit area (e.g., Longhurst, 1964). From the quantitative results on taxonomic composition of the benthos samples, together with corresponding results on environmental conditions, (e.g., salinity, temperature, sediment texture, etc.) the community associations of the benthos can be analysed.

3.3.1 Sampling on Soft Bottoms

There are special requirements for quantitative sampling of marine benthos on soft bottoms compared with chemical sampling of sediments. To obtain representative estimates of macrofauna species distributions, a larger sampling area than that required in physico-chemical analyses is needed (0.1 to 0.2 m^2 per sample), ideally to a depth of 30 cm, with no change in cross-sectional area with depth in the deposit (Holme and McIntyre, 1971). In addition, three to five replicate samples (for samples with cross-sectional areas of 0.1 m^2) are recommended to be taken at random within the area of the station (Holme and McIntyre, 1971, 1984; Stirn, 1981) to assess variability and collect a high proportion of the species present.

The conventional means of obtaining quantitative samples of benthos on soft bottoms is by grab sampler. There are many types of sampling gear for macrofauna available (for reviews see Section II-2.1 of this report; Holme and McIntyre, 1971, 1984; Thorson, 1957a; Holme, 1964; Hopkins, 1964). Small grabs commonly used in studies of freshwater benthos, such as the Ekman, Ponar and Number 1 orange-peel grabs are not recommended for sampling marine macrofauna. These grabs are not very effective for collecting deeper burrowing infauna. Also, the samples obtained by these grabs are so small that large numbers of replicate samples would be required to get reliable estimates of even the shallow burrowing marine fauna.

In using grab samplers for benthos studies it is often assumed that the instruments catch all of the organisms under the surface area covered by its jaws. A number of studies on the effectiveness of various grab samplers, however, have shown considerable variation in sampling characteristics between grab types. Gallardo (1965) compared the biting profiles in hard and soft substrata of three 0.1 m^2 bottom samplers: the Petersen, van Veen and Smith-McIntyre grabs. Examination of grab bites indicated that although the Petersen and van Veen grabs both dig deeper along the edges of the bite, the van Veen grab made the most rectangular cuts in both types of bottom. For the Smith-McInytre grab, the consistency of the sediment affects the shape of the bite, with the bite profile being deeper in the middle in soft substrate and less deep but more rectangular in firm sand. In the first two samplers, the bite profiles appear to be a result of their particular leverage-based closing mechanisms. Gallardo (1965) estimated that both Petersen and van Veen grabs will usually have greater than 50% efficiency in removing sediment from a bite of a given maximum depth, with that of the van Veen approaching 100% due to its more nearly quadrangular bite. The Smith-McInytre grab has slightly less efficiency than the other two grabs, but it catches more sediment in a given substrate due to its deeper bite resulting from a more powerful closing action.

Numerous attempts have been made to compare the effectiveness of various 0.1 and 0.2 m^2 grab samplers through measurements of biomass and benthos population density (Thamdrup, 1938; Ursin, 1954; Smith and McIntyre, 1954; Birkett, 1958). However, sampler comparisons suffer from the fact that the abundance of natural populations of benthos often varies widely even over small areas, complicating the calculation of the sampler efficiency. Nevertheless, field comparisons such as these made over a range of sediment types and weather conditions, together with analyses by movie camera observations of grab operation in tanks (Wigley, 1967) have revealed the following operating characteristics of the commonly used grab samplers:

(a) The original Petersen grab (Petersen, 1913; Davis, 1923, 1925) operates satisfactorily in soft, level bottoms but has lower penetration in fine or compacted sand than the van Veen and Smith-McIntyre grabs (Thamdrup, 1938; Ursin, 1954; Smith and McIntyre, 1954; Birkett, 1958). Much of the improvement of the performance of the van Veen over the Petersen grab is likely due to the greater leverage exerted by the long arms in closing the jaws. This leverage is greatest when the van Veen is fully open and decreases as the jaws close and the arms come together (Smith and McIntyre, 1954).

(b) The Smith-McIntyre grab tends to penetrate deeper in firm substrates and obtain higher population estimates than the Petersen and van Veen grabs (Smith and McInyre, 1954; Wigley, 1967). The deeper penetration achieved by the Smith-McIntyre grab is likely a result of a leverage affect which increases as the sampler digs into the sediment and reaches a maximum as the jaws come together. The introduction of metal screens covering the top of the bucket of the Smith-McIntyre sampler reduced the shock wave preceeding the grab during lowering and thus reduced avoidance by surface-dwelling macrofauna. Subsequent modifications of the van Veen grab greatly increased the screened window area on the upper side of the bucket to reduce the shock wave. Rubber flaps over the windows prevented loss of sediment during ascent. Addition of 20 kg of lead weights to the upper edges of the Petersen and van Veen grab jaws improved their penetration in firm substrates (Thorson, 1957a; Christie, 1975; Stirn, 1981).

(c) The Smith-McIntyre grab operates reliably in heavy seas or deep water because the sampler must land squarely on its tripping pads before the release mechanism will operate. The van Veen grab, on the other hand, is liable to close prematurely in rough weather due to changing line pull. To improve reliability of closing, the chain-rigging and release mechanism introduced with the van Veen grab by Ursin (1954) should be used. To prevent premature closing in rough seas or deep water, the pilot weight tripping device introduced by Lassig (1965) can be used.

(d) Active epifauna often escape collection by grab and are normally not sampled (Steven, 1930; Thorson, 1957a; MacGinitie, 1939). This shortcoming of grab sampling may be overcome by supplementary techniques such as photography, dredging or trawling (see Section 2.3.1).

3.3.2 Selection of a Quantitative Remote Sampler for Macrofauna

Choice of an appropriate remote sampler for individual arctic benthos studies is a compromise between study objectives, logistics and operating conditions during collection. It is desirable, however, to standardise the basic sampling techniques where possible to improve comparability of benthos data. Some guidelines for selection of a remote sampler for arctic sampling are indicated below, based on general references such a Thorson (1957a), Holme and McIntyre (1971, 1984) and on practical experience.

3.3.2.1 Sampling with small boats or limited lift capacity in open water.

Quantitative remote sampling of benthos from vessels can be difficult if the vessel or deck equipment is too small to handle heavy gear. Holme and McIntyre (1971, 1984) and others (see Section 3.3.1) have considered that scaled-down versions of grabs (e.g., Ponar, Foerst, Ekman, etc.) are usually not very effective for capturing deeper burrowing infauna. If these samplers are chosen for ease of manual operation, then long series of hauls must be made to get a representative collection of shallow burrowing macrofauna (Thorson, 1957a). The 0.1 m^2 sampler size is about the smallest which will sample effectively according to most authorities (Thorson, 1957a; Holme and McIntyre, 1971, 1984; Stirn, 1981). The order of preference for remote samplers for use in open water from a properly equipped vessel (based on overall effectiveness) might be:

1. Smith-McIntyre grab, 0.1 m^2; weight 45 kg (e.g., Carey and Ruff 1977).
2. van Veen grab, 0.1 m^2; modified from the original version by improvements discussed in Section 2.3.2.1; weight 41 kg (e.g., Lie and Pamatmat, 1965).

There are other factors associated with arctic sampling, however, which tend to favour the van Veen grab for general use. The van Veen sampler is less bulky for shipment, less awkward to handle aboard ship, cheaper in cost, simpler in action and can be modified for sampling through the ice (Petersen, 1977) by shortening the length of the lever arms. Word (1976) compared the benthos sampling efficiency of seven remote samplers (Ponar, Corer, Shipek, van Veen, orange-peel, Smith-McIntyre and a chain-rigged van Veen) in the silty sand to clayey silt sediment off southern California. His results indicated that the chain-rigged van Veen grab is the most reliable remote sampler for these sediment types. This choice was also recommended for biological monitoring off California (Tetra Tech, 1982) and in the Mediterranean (Stirn, 1981). Personal experience with use of the chain-rigged van Veen grab in the Beaufort Sea has found it to be reliable in most sediment types, except gravel, where the performance of all grabs is compromised.

The following precautions and practices have been suggested for use of the 0.1 m^2 van Veen grab (Lie and Pamatmat, 1965; Christie, 1975; Petersen, 1977; Stirn, 1981):

1) The vessel should be anchored on station, if possible, to prevent drifting off position.

2) The setting down of the grab should be done as gently as possible, in order to reduce the shock wave. The immediate closing of the grab should prevent the loss of sediment by lifting the grab before complete closure. In shallow water, the grab can be closed by hand by pulling the wire up and down in small short jerks.

3) The wire angle should be kept as small as possible to ensure that the grab strikes the bottom squarely and lifts vertically. Sampling while at anchor for open water studies generally achieves this condition except in areas with strong currents.

4) The volume of sediment should be measured in a graduated container and recorded. If less than 5 L of sediment is collected, as often happens on firm sandy bottoms, then the results should indicate that a low sample volume was obtained.

5) The exact sampling area of the particular grab should be carefully checked so that accurate conversions of results to per m^2 can be made. This can be determined by measuring the dimensions of the grab jaws or by performing land-based trials with the grab to permit measurement of the excavated area.

6) Adequate training and experience of personnel in the operation of the grab should be provided before actual field sampling commences. There is a "learning curve" for grab operation (Ursin, 1954; see Table 4.3 - Vol. 2)

7) It is important not to use too heavy wire for grab sampling since most grabs sink rather slowly. Wire which is too heavy may form a loop below the grab, possibly causing kinking and fouling. For similar reasons, the grab should be lowered steadily with gentle braking on the winch. The most convenient wire for the 0.1 m^2 van Veen grab is 4-6 mm diameter, of good quality and serviceability. Between the grab and the wire terminal a strong swivel should be connected to prevent twisting and kinking of the wire.

3.3.2.2 Sampling from the ice

General considerations for sediment sampling from the ice are reviewed in Section II-7.1. Particular aspects of remote sampling of benthos from the ice which affect the choice of sampler are discussed here.

Grab sampling from the ice is a compromise between obtaining a series of representative (i.e., 0.1m^2) samples and using the easiest prepared hole in the ice and the most portable equipment. For this reason it is not usually practical to use a grab with a bulky frame such as the Smith-McIntyre sampler. Among the types of grabs which have been successfully used in benthos studies from the ice in the Arctic are the 0.07 m^2 Foerst grab (Ellis, 1960; Curtis, 1972), the 0.1 m^2 Petersen grab (Thorson 1946) and a modified short-armed 0.1 m^2 van Veen grab (Petersen, 1977). A smaller, 0.023 m^2 box sediment sampler (the Birge-Ekman grab; Erickson _et_ _al_., 1983) was used from the Beaufort Sea ice in a combined geochemical and biological sediment sampling program.

Special procedures for benthos sampling through holes in the sea ice have been described by Petersen (1977). Winter grab sampling near Greenland was done with a hand winch mounted on a portable stand. A metre-wheel placed at the top of the stand also served as the tackleblock. To reduce the height of the stand and the size of the ice hole, a van Veen grab with 50% shorter arms was used, with no apparent loss in performance under the sediment conditions encountered. Usually two and sometimes three grab samples were taken through each hole in the sea ice, keeping the wire in opposite corners of the hole for each sample to prevent overlapping of grab bites. Benthos samples were sieved in sea water, even at air temperatures below -30°C. This method was found to be better than wrapping the samples in sackcloth and letting them freeze solid for transport to the laboratory where they were thawed and sieved in warm fresh water. The latter method damaged the specimens. Sieving at temperatures much below freezing will not usually be practical, however, because of icing of the sieve.

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4. DIRECT SEDIMENT COLLECTION AND OBSERVATION

4.1 Diving

The development of the aqualung (SCUBA), light helmet gear for surface-demand diving systems and improved insulated diving suits has led to an increased application of diving methods for direct observation and sampling in shallow water studies of aquatic biology and related disciplines. Techniques of conducting underwater research through diving have been reviewed by Riedl (1963, 1967, 1980), Scarlato et al. (1964), Clark (1965), MacInnis (1966), Peres (1966), Jones (1971) and Gamble (1984). Several benthos studies in the Arctic have used diving as an integral investigative tool (Golikov and Scarlato, 1973; Matheke and Horner, 1974; Chapman and Lindley, 1980; Thomson, 1982; Dunton et al., 1982; Heath et al., 1982 a, b).

The use of submersibles and underwater vehicles has extended the limits of direct observation and collection to great depths in the oceans (Peres, 1960; Shepard, 1965a, b; Shepard et al., 1964). In the Arctic Ocean, the Canadian submersibles Pisces I and Pisces IV have been used for biological observations (Milne et al., 1969; Wacasey, 1975).

4.1.1 General Application

Diving excels as a technique for investigating the macrobenthos of hard or gravelly substrates, especially steep rock slopes and cliffs, boulders and uneven surfaces or caves and canyons. Under these conditions, sampling by divers is superior to remote sampling. On level, soft bottoms diving can also be used, but it has fewer advantages. In situ growth, recolonisation and behaviour studies on attached benthos can only be made by diving (Baird, 1958; Limbaugh, 1961; Altman, 1967; Chapman and Lindley, 1980; Dunton et al., 1982; Heath et al., 1982a, 1984a). Diving observations can also provide valuable insights into the distribution of epibenthos and the interactions between organisms and their environment. Hand collection by divers can be an effective means of obtaining specimens in good condition for laboratory studies or identification.

The main limitation to scientific diving is the depth at which a diver can carry out a long series of tasks, as opposed to cursory observations or collections. Unless special equipment is used the limit is about 35 m, but a safer limit for lengthy dives is 20 m without providing for decompression stages. When working in isolated areas without a decompression chamber on site, it is advisable that diving be performed within safe no decompression limits (see Shilling et al., 1976, for details).

Tasks which can be performed by divers are varied, including still photography, underwater video recording, visual observations, quantitative sampling (see Section 3.1.3), hand collection, and measurements for growth, recolonisation or behaviour experiments. Handling equipment in cold water can be more awkward than on shore, and sustained hard physical work is difficult to perform underwater. The relative buoyancy of many objects in water, however, can be an advantage for divers. The use of diver communications and an assistant on a dive tending vessel for lowering and raising equipment when required can greatly increase the effectiveness of diver sampling since there is a limit to the gear a diver can carry during a dive.

In turbid water, or on bottoms with fine deposits which easily become disturbed, poor visibility can cause difficulties in performing tasks or in recognising features on the bottom. Further constraints to diving are rough seas which jeopardize safe entry and exit from the water, and proximity to high concentrations of drifting ice floes, which might prevent safe anchoring of the support vessel or endanger the diver. At all times the diver's safety should be of paramount concern.

4.1.2 Diving Gear for Arctic Applications

The frigid waters of the Arctic Ocean are a major factor to be contended with in arctic diving. Surface heat losses can be greatly reduced by use of good quality dry suits, woolen long underwear and 9.5 mm thick neoprene diving mittens. However, diving suits with hot water circulation provided through an umbilicus offer the best temperature control in frigid water applications of the surface demand system (Gamble, 1984).

Air supply can be from SCUBA (self-contained open-circuit underwater breathing apparatus) or surface-demand supply. In the surface demand system, the breathing mixture (compressed air) is supplied to the diver by an air hose part of the (umbilicus) directly from a low-pressure compressor and volume tank or from a regulated high pressure source (e.g., K-bottles). For maximum protection and comfort the diver can wear a light fibreglass head-mounted diving helmet that moves with the diver's head and keeps the entire head dry (e.g., Oceaneering "Rat Hat" or Kirby-Morgan "Superlite -17" Diver's Helmet). These helmets are equipped with diver-to-surface communications, demand regulator, reserve air supply and many other safety features. Less sophisticated full face mask equipment is also available (e.g., Kirby-Morgan band mask). Only one diver is required in the water when the surface-demand system is used; a second diver is "dressed-in" on standby. A diving supervisor operates the equipment on the surface and co-ordinates the diving operation. This system provides an ample air supply for the diver and for airlift sampling during a lengthy dive. If a precautionary decompression stop is made, there is no concern about an adequate air supply.

The surface-demand system is standard for commercial diving and is highly suited to cold water applications in remote areas such as the Arctic. This system was used successfully in depths of 3 to 20 m during benthos surveys at marine gravel dredging sites and around an artificial offshore island in the Beaufort Sea (Heath et al., 1982a, b; Heath and Thomas, 1983; 1984a, b). Angel and Angel (1967) described marine benthos sampling at 35 m near Hollandervik, Norway, which was performed by divers using surface air supply.

A commercial surface-demand diving system (for 1 diver + standby) costs about $15,000 to $20,000, but can be rented in the Arctic for $250 - $300 per day.

The SCUBA system requires less equipment in the field provided that an adequate supply of diving tanks is available. For diving in remote areas or under ice, each diver should be equipped with a primary and reserve breathing system and headphones for wireless underwater communication with attendants on the surface. (e.g., UDI-SUBCOM, Aberdeen, Scotland). Divers are tethered in tandem to the surface by lifelines for under-ice dives or in areas with significant currents. Under no circumstances should decompression stops in the water be required for completion of a routine dive with SCUBA.

Studies of arctic macrobenthos involving use of SCUBA for sampling have included seasonal growth studies of kelp at Igloolik, N.W.T. (Chapman and Lindley, 1980), the Baffin Island Oil Spill Project (BIOS, Cross and Thomson, 1981), a survey of benthos in Lancaster Sound and northwest Baffin Bay (Thomson, 1982) and a study of an arctic kelp community in Stefansson Sound in the Alaskan Beaufort Sea (Dunton et al., 1982). Soviet research on arctic benthos has also used quantitative SCUBA diving techniques (Golikov and Scarlato, 1973; Golikov and Averincev, 1977) to depths of 40 m.

The purchase cost of SCUBA diving equipment for cold water ($2,500 -$3,000 per diver) is considerably less than commercial surface-demand diving systems. However, since air stations are scarce in the Arctic, high pressure compressor(s) would be needed for refilling dive tanks. It should be noted that dive tanks can only be air-transported when empty.

Diving through the ice requires a suitable dive hole which can be cut by a variety of means (see Section II-7.2). The large blocks may have to be lifted by helicopter or multi-terrain vehicle (e.g., Dunton et al., 1982). A heated, insulated tent (e.g., Parcoll, Panebec Ltd. Montreal, Hansen or Norseman tent) can be placed over the dive hole to act as a dive shelter and field laboratory. When ambient underwater light is low or absent, strong diving lights will be required.

4.1.3 Positioning

Navigation aids for locating dive stations are generally supplied by the support vessel during the open water season. For highly accurate fixes a shore-based navigation system such as a Decca Mini-Fix or Del Norte Trisponder system may be appropriate. To mark dive sites which must be revisited exactly, an acoustical pinger placed on the position and a directional receiver on the surface or held by the diver may be used (e.g., Helle or Hydro-Products). Buoyed floats or other surface markers are prone to be removed by drifting ice. If left over winter, the bottom mounted pinger should be deployed in an area protected from ice scouring (e.g., Dunton et al. 1982). Supplementary surface mounted flags can be used in winter in areas where ice movement is minimal.

4.1.4 Diver-Operated Sampling Techniques

In addition to its advantages for direct observation and hand collecting of benthos specimens, diving has considerable utility for direct quantitative sampling of benthos and sediments for chemical analyses as well as still photography and video recording.

4.1.4.1 Quantitative Benthos Sampling by Divers

a) Line Transects

On hard substrates, quantitative estimations of benthos may be performed along line transects with sampling intervals marked on a rope or tape (Kain, 1962; Knight-Jones et al., 1967; Cross and Thomson, 1981). Lines for transects may be stretched between permanent stakes if an area is to be re-sampled (Cross and Thomson, 1981), or divers may leap-frog with a line along a compass heading to cover fairly long distances in an underwater survey (Kain, 1962). A weighted line ("lead line") is a convenient form of underwater marker for line transects. Anchor lines to surface floats are useful for checking orientation of the transect and for ease of location by the divers when descending. Gamble (1984) has described applications of transects for in situ benthic studies.

Sampling techniques which may be used in conjunction with line transects are numerous; these include:

1) in situ counting and supporting collections of large organisms over prescribed intervals (Forster, 1959; Larsson, 1968);

2) quadrat sampling at randomly selected intervals by scraping into a net bag (Kain, 1960), or airlift sampling (Barnett and Hardy, 1967; Cross and Thomson, 1981; see also Part b of this section); and,

3) quantitative diver-operated still photography within a fixed frame (Cross and Thomson, 1981; Dunton et al., 1982, see Part c of this section).

In situ counts along transects may be necessary for invertebrates and macroalgae which are too large and sparsely distributed to be representatively sampled by quadrat sampling or photography. For example, counts of organisms such as starfish, sea urchins or macrophytes can be made in one meter wide strips 10 m or more in length by a diver holding a 1 m rod before him, keeping one end of the rod above the transect line and counting the organisms as it passes over them (Forster, 1959; Larsson, 1968). Collections of representative animals and plants of uncertain identity should also be made for species identification (Cross and Thomson, 1981).

b) Quadrat Sampling

The smaller sessile and slow-moving benthos can be quantitatively sampled within quadrat frames of various shapes and sizes; the smaller and more abundant the species to be counted, the smaller the appropriate quadrat. Gamble (1984) has reviewed the minimal area concept for diving studies. There is general agreement that the minimal sampling area is that which, if doubled, would yield only a 10% increase in information (eg. Weinberg, 1978). Species-area curves can be prepared from results of a series of quadrats.

On hard bottoms, the benthos may be removed by a scraper with a net bag attached (Kain, 1960) or by scraper and airlift sampler. In particular, the Finnish IBP-PM Group (1969) have described a suction sampler for hard bottoms which isolates the quadrat area (0.11 m^2) from the surrounding area with an inverted plexiglass funnel which is connected to an airlift (Figure II-4.1) operated from a surface air supply (compressor or bottled air). The funnel has a plastic foam seal around the bottom edge and small openings covered with fine (0.5 mm) screens through which water can freely pass. A pair of rubber gloves connected to the upper sides of the funnel permit manipulations such as scraping to be done inside the funnel. The benthos detached from the rock surface are sucked up the airlift tube and retained by sieves or nets on the raft at the top. This system is suited to depths of 2-15 m and generally requires two divers and a surface tender for effective operation. The apparatus is usually bulky, but can be separated into sections for transport and storage. The sampler can be positioned and moved for short distances by the divers, who need not ascend before the sampling is completed. In rough weather the raft must be anchored to relieve strain on the suction pipe, which will separate at the joints. This suction sample is also limited to relatively flat surfaces of rock or large boulders. It does not work well on irregular stony bottoms (Finnish IBP-PM Group, 1969) because the funnel will not seal on the substrate. Despite these limitations, the Finnish suction sampler is a highly effective quantitative apparatus for shallow areas with hard, level substrates, especially where algae occur. It may be useful for sampling such zones in the eastern Canadian Arctic.

On soft bottoms, the advantages of diver-operated sampling are less obvious, especially for the infauna, but divers can make valuable observations, select appropriate sampling sites where species distribution change abruptly, and obtain deeply burrowing animals which might otherwise be missed (Barrett and Hardy, 1967). Quadrats are as useful on soft substrates as on hard ones for quantitative estimates of larger epifaunal plants and animals (Jones and Kain, 1964). To sample the infauna, diver-assisted sampling by various designs of corers or box samplers or light grabs (e.g., Ekman), may be used (e.g., Fager et al., 1966; Angel and Angel, 1967; Finnish IBP-PM Group, 1969; Harper et al., 1980).

Divers may also sample infauna from soft sediments with suction devices such as those described by Brett (1964), Masse (1967, 1970), Reys et al., (1966), Barnett and Hardy (1967) and Emig and Lienhart (1967). When combined with a quadrat frame or cylinder, these devices may be used to collect quantitative samples.

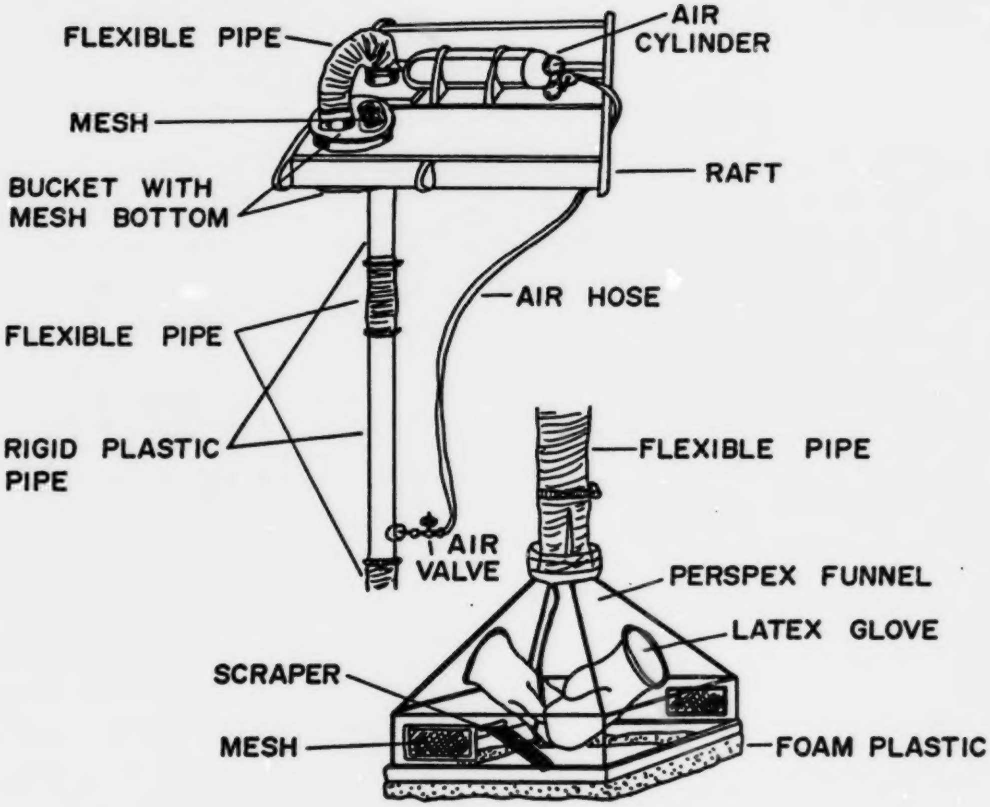
FLEXIBLE PIPE
AIR CYLINDER
MESH
RAFT
BUCKET WITH MESH BOTTOM
AIR HOSE
FLEXIBLE PIPE
RIGID PLASTIC PIPE
FLEXIBLE PIPE
AIR VALVE
PERSPEX FUNNEL
LATEX GLOVE
SCRAPER
MESH
FOAM PLASTIC

Figure II-4.1. Finnish suction sampler for hard bottom (redrawn after Finnish IBP-PM Group, 1969).

The airlift principle employed by the diver-operated samplers of Barnett and Hardy (1967), and the Finnish IBP-PM Group (1969), has been widely used in various forms in benthos studies involving diving. The Barnett and Hardy sampler uses suction both to assist in driving a coring tube into the sediment of level soft bottoms, and to remove the sediment and infauna from the 0.1 m^2 area contained by the 60 cm long cylinder (Figure II-4.2). Initially, the cylinder is worked into the sediment by the head of water over the lid of the partially evacuated cylinder. After full penetration of the cylinder, the lid is opened and an airlift pipe (8 cm diameter, 3-6 m long) is used to suck the contents up into an enclosed sieve (1 mm metal mesh) to which is attached a polyethylene sample bag. The device is most effective in firm sediments at water depths of 3-10 m, but can be used to somewhat greater depths (Barnett and Hardy, 1967). General modifications of the original design have been devised, generally employing a self-contained airee supply for SCUBA applications, rather than the surface air supply (eg. Keegan and Konnecker, 1973). These workers also used a stainless steel coring cylinder (0.1 m^2 surface area) with a bevelled lower edge and circular hand grip at the top to enable the cylinder to be driven manually into the sediment. They found this cylinder system, which is similar to that of Masse (1970), to be less time consuming for use in coarser sediments than the suction driven cylinder of Barnett and Hardy (1967).

Diver operated sampling in depths of 3-20 m in Baffin Bay, Lancaster Sound and Eclipse Sound was performed with a 2 m long airlift sampler consisting of 8 cm diameter weighted PVC pipe, fitted with a 20 mPa air cylinder and a first stage dive regulator that delivered a constant pressure of 860 kPa above ambient pressure, (Cross and Thomson, 1981; Thomson, 1982). Quick release fittings on the 1 mm mesh sample bag enabled it to be removed from the top of the suction pipe and capped. Triplicate sampling was performed within three sample rings in a triangular array; they were equipped with 1 mm mesh trap nets to isolate the animals within (0.06 m^2, Cross and Thomson, 1981; 0.15 m^2, Thomson, 1982). The trap nets each contained a quick release fitting to connect with the suction pipe. The airlift penetrated to depths of 2-25 cm, usually 5-15 cm.

Airlift sampling was also performed during benthos surveys in 3-18 m depth near Herschel and Banks Islands in the Beaufort Sea (Heath et al., 1982a; 1982b; Heath and Thomas, 1984a; 1984b). An airlift tube (6.4 cm diameter, 2 m length) equipped with a thread-on 1 mm mesh sample net and quick release connection to a surface air supply (860 KPa above ambient pressure) was used to excavate sediment and infauna within a 0.25 m^2 quadrat frame in substrates ranging from clay to gravel.

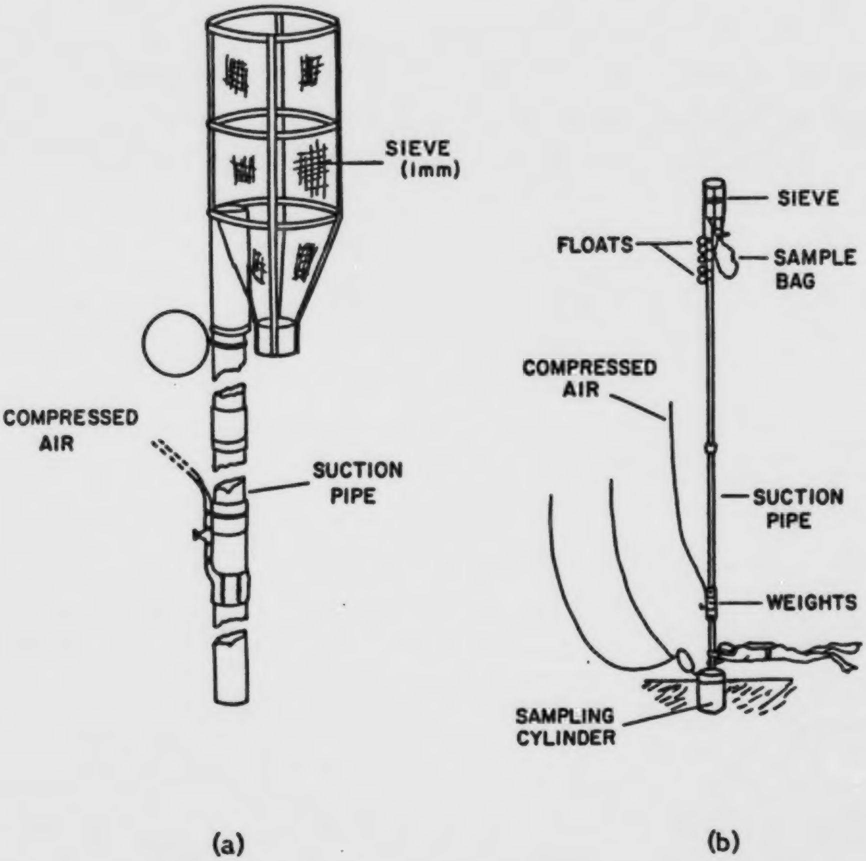
SIEVE (1mm)
COMPRESSED AIR
SUCTION PIPE
(a)
SIEVE
FLOATS
SAMPLE BAG
COMPRESSED AIR
SUCTION PIPE
WEIGHTS
SAMPLING CYLINDER
(b)

Figure II-4.2. Barnett and Hardy air lift sampler
(a) Detail of suction pipe and sieve.
(b) Method of operation. The lid of the sampling cylinder is opened and the sediment is drawn up the vertical suction pipe into the sieve. The residue falls from the sieve into the attached plastic bag (redrawn from Holme and McIntyre, 1971).

Penetration was generally from 5-10 cm. A total area of 0.5 to 1.5 m^2 was sampled at each site.

Other diver-operated samplers generate suction by aspiration (Brett, 1964; Masse, 1967; 1970), or propeller action (Emig and Lienhart, 1967). Brett's sampler is powered by a 3 h.p. engine at the surface which pumps water down a pipe inserted axially into the collecting tube. A coarse mesh (3.2 mm) on the collecting bag is necessary to prevent clogging. This sampler can excavate the depths of 20-30 cm, but unless the metal frame quadrat is driven deep enough, the sides of the hole will cave in. The aspirator sampler of Emig and Leinhart has a similar suction pipe to that used by Brett, with filtration through a rigid cone of metal screening. Suction is generated by a propeller driven by a self-contained 12 volt motor and dry cells within the body of the sampler. For quantitative sampling, a 40 mm diameter tube is used to extract the contents of a cylinder which has been forced into the bottom. Emig and Leinhart's sampler is more easily handled than devices which have surface connections, but is limited by battery life, especially in cold water, and by the coarse filter mesh size, a serious deficiency if smaller organisms are abundant. Large infaunal specimens or rocks are also liable to plug the narrow intake tube.

Each of the diver-operated samplers described in this section has some advantages and disadvantages. For sampling level, hard substrates in shallow water, the Finnish suction sampler is likely the most effective quantitative sampler. The Barnett and Hardy (1967) sampler appears to be the most thorough quantitative sampler on shallow, firm sandy bottoms. For sampling areas in which both bottom types occur, it would be desirable to be equipped with the 0.1m^2 cylinder of Barnett and Hardy (1967) or Masse (1970) and the Finnish suction sampler. The air lift from the latter device could then be used with the coring cylinder on sandy or soft bottoms.

c) Still Photography and Underwater Television

The use of hand-held diver-operated cameras is the simplest application of underwater photography and television. In shallow water (under 30 m), good results are more easily obtained by a diver than by remote equipment; a diver cameraman can also operate in places which are inaccessible from the surface. Video and photography can provide a permanent record of underwater observations and to capture rapid events which could otherwise go unnoticed (Edgerton, 1963). These properties can be very useful in studies of growth and colonisation of sessile benthos and in description of the distribution and movement of fauna.

A photographic recording technique to make the most of photographic opportunities on each dive has been developed by Johnston et al., (1969). They have also shown how accurate plots of individual organisms can be made by using a calibrated marker frame included in the photograph.

When making quantitative analyses from serial photographs, it is important that every relevant frame should be included in the study in order to avoid over-emphasis of a small number of particularly interesting pictures (McIntyre, 1971). Photographs should be examined in concert with benthos collections from the area whenever possible. This is often more practical in diver-operated photography than in remote photography. Holme (1984) has recently reviewed many aspects of diver and remotely operated photography and television for benthic studies.

Cross and Thomson (1981), combined quantitative photography with line transects in a study of macrobenthos near Cape Hatt, Baffin Island. A photographic record of each transect was made on colour slide film with a Nikonos camera equipped with a 15 mm lens, paried Vivitar electronic flashed and a fixed focus framer covering a bottom area of approximtely 0.5m^2. Ten photographs were taken at randomly positioned intervals along each transect. The photographs were used to estimate densities of visible epifauna that were too sparsely distributed to be representatively sampled by airlift.

Underwater television and still photography were used in underwater surveys of the impacts of marine gravel dredging on benthos in the Beaufort Sea (Heath et al., 1982a, 1982b; Heath and Thomas, 1984a, 1984b). The photographic and video records provided valuable visual information in the assessment of impacts on epifauna and benthic habitat. These techniques effectively recorded the distribution of sparse and aggregated sessile epibenthos which were often associated with scattered rocks and boulders. They also documented the physical changes to habitat in dredge trenches and ice scours, and qualitative evidence of recolonisation by benthos in the trenches.

4.1.4.2 Diver-Assisted Sampling for Sediment Chemistry

A basic requirement of sampling sediments for chemical parameters is to obtain undisturbed and uncontaminated sediment, especially from the sediment-water interface. The use of diver-assisted samplers for obtaining undisturbed sediment samples has several advantages over remote samplers in this regard. When visibility and depth permit, a diver is able to:

1) select a suitable bottom area for sample collection if a particular sediment type is required;

2) operate a corer or grab sampler to ensure minimal disruption of the surface layer, adequate penetration into the sediment, and proper closure of the sampling device; and,

3) take visual observations or photographs of sediment conditions (such as ripple marks, ice gouges, slumping etc.), which aid in interpretation of particle size and chemical analyses.

Diver-assisted sediment sampling devices suitable for chemical studies include the Ekman and Birge-Ekman grabs, simple corers, and core tubes or boxes which can be sealed at both ends (e.g. Mawhinney and Bistrutti, 1981; Harper et al., 1980).

4.1.4.3 Ancilliary Diving Equipment

Devices which can improve the mobility or range of a diver include various diving sleds and planes which may be towed behind a boat and maintained either on or above the bottom by a single diver, (Sand, 1956; Hold, 1960; Riley and Holford, 1965; Erwin, 1977) or two divers (Sigl et al., 1969). These are valuable for surveys covering large areas, for mapping dominant features or for searching. Commercially produced self-propelled towing vehicles with diver controls are also available and are preferable for use in the vicinity of underwater cliffs or outcroppings of rock. Earll (1977) described primary survey strategy.

4.2 Submersibles and Underwater Vehicles

Submersibles and remote controlled vehicles can be used for the study of benthos beyond the depth range of divers (eg. Heirtzler and Grassle, 1976; Geyer, 1977). Early designs lacked well developed methods of taking and manipulating samples in sufficient numbers (Wischhoefer and Jones, 1968), but newly developed manipulator arms are overcoming this problem. The cost of underwater research vehicles, however, is relatively high for most projects. The purpose of this section is to indicate what underwater vehicles are available for use in the Arctic. Although their primary purpose is likely to be for offshore petroleum exploration or national defence projects, there may be opportunities for observations on benthos to be made if researchers are aware of the possibilities.

Submersibles range in size from large submarines to one atmosphere diving suits (ADS). Canadian federal government agencies have operated research submersibles ("PISCES I" and IV) in arctic waters. Milne et al. (1969) described observations on the usefulness of the "Pisces I", a two-man sub for depths to 610 m, as a research tool deployable from an icebreaker in the Arctic. Underwater work performed in open water and under sea ice included under-ice acoustics, exploration of bottom topography, collection of sediment, rocks and benthic organisms, in situ salinity sensing, underwater photographic and video recording of benthos and geological features and direct scientific observation. Wacasey (1975) reported on observations made from the "Pisces IV" submersible on the density and distribution of epifauna in Thetis Bay, near Herschel Island, Y.T. Density estimates were based on the mean number of epifauna observed within a 1 m^2 metal frame held near the bottom by the manipulator arm of the submersible for a number of trials.

The operations of small submersibles launched and recovered by motherships are limited by rough seas (>1 m), high turbidity, and proximity to ice. Diving support services to the frontier offshore petroleum exploration industry have developed a variety of manned and remote controlled underwater vehicles. Examples include the one-man ADS systems named "JIM", "JETJIM", "WASP" and "WASP Inspector", the one-atmosphere diving bells, "ARMS I" and II, the remotely operated vehicle (ROV), "SCORPIO", and the submersibles "DEEP ROVER" and "PISCES IV". Many of these submersibles have force-feedback manipulator systems (e.g., General Electric "ARMS" manipulator) for a high degree of responsiveness and control even in limited visibility. They are also equipped for video and still photography. The ADS systems are limited to 610 m; the bells and ROV's have been used to 915 m, and the submersible "PISCES VI" is rated for a maximum depth of 2515 m (Nellessen, 1982). Several of the above systems have been used by Can-Dive Services Ltd. in support of deep water drilling operations in the eastern Canadian Arctic. Opportunities may arise for collections or observations of deep water benthos during inspection dives on drill rigs. The high cost of leasing submersibles ($2,000 to $20,000 per day) is prohibitive for most research projects.

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5. METHODS FOR SAMPLING PHYTOBENTHOS

5.1 Introduction

The marine phytobenthos, in the broadest sense, includes all primary producing organisms in the oceans and seas (bacteria, algae, lichens and angiosperms). With special reference to the Arctic, this review will consider the benthic marine algae, which are responsible for the greater part of primary production in the marine environment. Round and Hickman (1971; 1984), have presented a review of methods for phytobenthos sampling and estimation of primary production. A general reference on phycological methods is Stein (1973).

5.1.1 Arctic Marine Algal Habitats

In order to compare algal communities and primary production in different areas, it is necessary that habitats and algal associations are well defined and standardised methods are used. The term "association", refers to 'an assemblage of species that recurs under comparable ecological conditions in different places' (Hutchinson, 1967). The recognition of 'comparable ecological conditions' is simplified if the habitats are clearly described (Round and Hickman, 1984). Habitats can be defined according to physico-chemical characteristics without reference to the organisms. The nature of the substratum should be clearly described along with any information on slope angle slope aspect, length of tidal exposure, and degree of exposure to wave action or currents. Environmental conditions such as temperature, salinity, irradiance, nutrients and depth or ice movements etc., are also important. Lee (1973) has described general environmental conditions affecting benthic marine algae in the Canadian Arctic. Wilce (1959) and Ellis and Wilce (1961) have provided ecological descriptions of intertidal algal communities in the southeastern Canadian Arctic.

In arctic Canada, large tides and waves usually occur in local areas and are generally seasonal due to ice conditions. The largest tides occur in Hudson Strait and on the southeast coast of Baffin Island; mean amplitudes range from 3.1 to 9.2 m. Western Hudson Bay (to 4.2 m) and eastern Ellesmere Island (to 2.9 m) also have significant tidal ranges, whereas most of the central and all of the western sections of the Canadian Arctic have small mean tidal amplitudes (0.03 to 1.0 m). Wave-induced turbulence in shallow water also tends to lessen in a westward direction, primarily due to increasing amounts of ice cover (Lee, 1973).

Inorganic substrata for arctic algae include hard or rock surfaces, soft or mobile sediments, and ice. In arctic Canada exposed bedrock and large boulders in the marine environment are mainly confined to the region bordered by Baffin Bay and Hudson Strait. Unconsolidated, small-sized material, make up most of the sea bed: cobbles, pebbles and, notably near fresh water discharge, sand and silt or clay (Lee, 1973). The deltaic areas of most freshwater outflows are characteristically muddy. Since these areas have no attached algal growth, their extensive and frequent occurrence tends to isolate the macroscopic epilithic ("growing on rock") algal communities to the deposits and outcroppings of rocky material.

Algae which inhabit soft or mobile inorganic substrata include those (episammic) attached to sand particles and probably shell fragments. There are also algae (epipelic) which move on and in silt and sand surface sediments.

Organic substrata which support attached phytobenthos include some microscopic and most macroscopic algae. The attached plants are referred to as "epiphytes"; the host plants are either attached to hard substrata, or form viable unattached communities on silt and clay bottoms (see Lee, 1973 for arctic examples). Algae may also be epibiotic on epifauna (i.e., epizoic) especially sedentary animals (e.g., gastropods, barnacles) associated with hard substrata.

The number of associations recognized in a given study will generally increase as detailed research proceeds. They are, in the narrow sense, definable only from the dominants, subdominants and other species present (Round and Hickman, 1984). They can however, be classified by the niches or micro-habitats which they inhabit within the gross habitats defined above. Round (1981) has provided further detail on this approach.

For environmental monitoring or impact studies on phytobenthos it is important to realise that due to the heterogeneity of algal associations in the littoral zone, it is not feasible to characterize well areas other than small reference sites, on the order of m^2, rather than km^2. Sampling programs for impact studies, therefore, should concentrate efforts within manageable areas which provide comparable environmental conditions for impacted and reference sites. Sampling designs for environmental studies on marine benthos are considered in more detail in Section 5, Volume 2.

5.2 Field Sampling Techniques

Before sampling can be performed, it is necessary to recognise the algal associations involved and the habitats (or micro-habitats) which they occupy (see Section 5.1). Where separation of mixed associations in the field is impossible (e.g., ephiphytes on macroalgae) the extent and heterogeneity of the associations should be recognized (Round and Hickman, 1971; 1984). Preliminary sampling is often needed to determine these factors and to guide the selection of sampling sites.

When planning field projects, especialy in isolated areas, it is important to recognise that *in situ* biomass and production studies may present complex logistical problems. Examples of such *in situ* studies where arctic sampling logistics were overcome, however, are described by Chapman and Lindley (1980) and Dunton *et al.* (1982).

Many of the methods of analysis for phytobenthos require examination of the taxonomical composition of the algal component of samples. This work is very time consuming and requires expertise to be done properly. These factors should be considered in planning studies involving phytobenthos.

5.2.1 Macroalgae and Other Epilithic Algae

5.2.1.1 Macroalgae

The density, biomass and distribution of macroalgae can be quantified by line transect sampling which combines *in situ* counts, quantitative photography and collections for quadrats (e.g. 0.25 m^2) for identification and biomass determination (Cross and Thomson, 1981; Dunton *et al.*, 1982). Seasonal growth studies on the kelp, *Laminaria solidungula*, which involved repeated measurements on individual plants by divers during open water and under ice cover were conducted near Igloolik Island, N.W.T. by Chapman and Lindley (1980) and in Stefansson Sound, Alaska by Dunton *et al.* (1982).

For laboratory studies on macrophytes, the plants must be removed, preferably in whole for anlaysis. Holdfasts must be included in the samples. Plants can be sampled completely for weight, volume, chemical composition or individual plants can be marked and growth measured directly (e.g., Chapman and Lindley, 1980). Sampling of epiphytes usually requires removal of the host plant to the laboratory. Since some are loosely adhering; samples should be taken with a minimum of disturbance. It is advisable to enclose the host plant in a sampling devise (e.g., plastic bag) while it is still attached under water (Round and Hickman, 1984). The species dislodged during transport and handling should be added to the biomass of epiphytes still attached to the host plant.

Macroalgae which are collected for identification may be mounted on herbarium paper and air dried in a plant press. The species composition and biomass of small epilithic organisms may be determined by scraping and airlifting all biota within quadrat areas (e.g., 0.05 m^2) on rock surfaces. Results may be expressed per m^2 of rock substrata rather than of the sea bed if rock cover is not predominant (e.g. Dunton et al., 1982).

5.2.1.2 Epilithic Microalgae (Periphyton)

Quantitative sampling of the periphyton (epilithophyton algae growing upon the surface of rocks) can be done with a stiff nylon brush (from a toothbrush) attached with waterproof glue to the piston of a 50 cc open-ended disposable plastic syringe(Stockner and Armstrong, 1971). The sampler should be placed firmly against the rock surface by a diver and the piston with the brush tip rotated several times to remove the algae. The piston should then be slowly withdrawn into the syringe cylinder to collect the loose periphyton. A glass plate or stopper should then be placed over the end to the syringe cylinder and the sampler inverted. This device samples an area of 5.1 cm^2, with satisfactory removal of loosely attached periphyton such as diatoms and with no discernable damage to the algal cells (Stockner and Armstrong, 1971). Replicate samples should be taken at each location and the contents of each sample placed separately in 100 mL wide-mouth jars. The samples can be divided into aliquots for algal cell counts and chemical analyses.

The subsample for quantitative cell counts can be examined live under a microscope (x 450) to make estimates of percentage composition of major algal gorups. Counts of live cells can be converted to a volume basis by using cell volume conversions (e.g. Nauwerck, 1963). The relative contribution of each algal group can then be expressed as a percentage of the total algal volume present in each sample (Stockner and Armstrong, 1971).

Samples for algal cell counts should be preserved and stained by addition of Lugols iodine solution (e.g. Lund et al., 1958) and stored in the dark. The addition of 5% formaldehyde is desirable for extended preservation since the iodine will gradually lose its preservative effect.

If diatoms are present, it is necessary to prepare material and mount it on glass slides for subsequent identification and enumeration (e.g. Stockner 1968a; 1968b; Eaton and Moss, 1966). For enumeration of diatoms on permanent slides it is desirable to view them under oil immersion (1000 x) using differential interference microscopy (e.g. Zeiss-Normanski optical instruments; Stockner and Armstrong, 1971).

Glass slide experiments can be used in sequential sampling programs for estimation of change in biomass and season succession of periphyton (Stockner and Armstrong, 1971). The glass slides, 2.5 x 7.5 cm, can be placed in replicate horizontally on the substrate at a desired depth. At each sampling time they should be removed, and the accumulated periphyton scraped off and put in a small vial with a few drops of 10% formalin. The algal material can then be filtered on a tared filter (e.g. Milipore HA 0.45 μM) oven-dried to constant weight at 105°C, weighed and corrected for weight of the filter. For prepration of slides of diatoms, the periphyton and filter should be macerated in concentrated nitric acid and prepared as indicated earlier (Stockner and Armstrong, 1971). Quantitative diatom counts in a vertical and horizontal transect of each slide should be made under 1000 x magnification and converted to a per cm^2 basis for reporting.

Ash-free dry biomass estimations of periphyton form duplicate slides can be made by scraping off the algae, filtering on tared filters, drying at 105°C and weighing, followed by burning in a muffler furnace at 500°C. Weighing of the ash fraction allows determination of the organic fraction by difference (weight loss on ignition = organic mater). See Section 6.2.1.3, Volume I, for details on the ashing procedure. Biomass estimates can be expressed as biomass (mg ash-free dry wt/m^2).

5.2.2 Benthic Microalgae from Sediments (Epipelon)

A significant portion of the total primary production of arctic estuarine and nearshore habitats may be contributed by benthic microalgae (Matheke and Horner, 1974). Methods for measuring biomass and production of benthic microalgae that are episammic ("attached to sand") and epipelic ("moving in and on surface sediments") have been described by Hickman and Round (1970), Leach (1970), Round and Hickman (1971; 1984) and Matheke and Horner (1974).

Sampling of soft sediments can be done with corers, in the intertidal and underwater. Cores taken by divers should be carefully inserted into the sediment to avoid water movement, sealed by rubber stoppers and carefully handled to avoid loss of surface algal forms. Wide cores (> 10 cm diameter) are best for undisturbed samples with no noticeable core shortening due to wall friction. With remotely-operated corers, the downwash or "bow wave" of the instrument will lead to some loss of the superfical layer of most sediments (Eleftheriou and Holme, 1984). The sampled area should be indicated in order to express biomass and production on an areal basis (per m^2).

Epipelic algae can be removed from damp sediments by placing a piece of fabric (e.g., lens tissue) on the surface and allowing time (usually 12 hours) for the phototactic algae to move up into it (Eaton and Moss, 1966).

The time of final harvesting of the algae before enumeration is important because epipelic populations exhibit strong diurnal vertical migration rhythms (Round and Palmer, 1966; Round and Hickman, 1984) in the field and under laboratory conditions. Since the movement is in phase with tidal cycles occurring at the same time in the field, the timing of the migratory cycle must be determined by preliminary sampling and harvesting time adjusted accordingly (Round and Hickman, 1984). Samples in the laboratory should experience a normal light/dark cycle. Undisturbed core samples of benthic micro-algae can be also treated in three other ways. After being resuspended in the laboratory to disperse the algae, the samples may be:

i) subsampled and enumerated in a counting chamber (Aleem, 1950; Hopkins, 1963);

ii) poured into dishes, with coverglasses placed on the damp sediment surface. The epipelic algae move up onto the coverglass which can be removed and placed on a slide for counting and enumeration (Round, 1953); or

iii) gravimetrically separated by ascensional water flows, followed by differential centrifugation in liquids of differing densities (Plante, 1966). This treatment is effective in separating diatoms and fine detrital material.

The first two techniques have been combined by Eaton and Moss (1966) in a quantitative method of defined accuracy for sampling and estimating epipelic populations under a variety of field conditions.

Episamnic algae are also collected by sediment corer. To achieve separation of the mixed benthic algal associations, the non-episamnic algae are removed by repeatedly swirling the sediment with filtered water. The episamnic flora remains attached to the sand grains which sediment out, while the epipelic algae and detrital material can be decanted off (Round and Hickman, 1971; 1984). The cleaned sand is then subjected to sonification to remove the episamnic algae from the sand for cell counts either directly from aliquots, or dried and slide mounted. The sediment used is dried so that all estimates of cell numbers can be related to the area and volume sampled (Round 1965; Moss and Round, 1967).

5.2.3 Estimation of Microalgal Biomass from Chlorophyll Extracts

An alternate but inferior method to enumeration of the density of algal cells for estimation of standing crop or biomass is the measurement of chlorophyll pigment concentrations (Moss, 1967a; 1967b). It has been demonstrated (e.g., Healey (1980)) that chlorophyll-a is not a good index of standing crop because its concentration in algal cells is a function of mean irradiance and nutrient concentrations. The concentration of chlorophyll-a per cell can vary by an order of magnitude with changes in nutrient concentrations (Healey, 1980). In addition, it is very difficult to measure chlorophyll-a adequately in benthic samples. The trichromatic equations of Strickland and Parsons (1968; 1972) do not apply well for benthic microalgae. The more complex techniques of thin layer chromatography or scanning spectrophotometry (Stainton, 1973) are needed for satisfactory measurement of chlorophyll-a in benthic algae.

5.2.4 Estimation of Primary Production

Algal primary production can be estimated in a number of ways, the simplest and most direct method being by the change in biomass (volume, length, or weight) over a measured time interval (see Section 6.2.1). This approach is suitable for studying the production of macroscopic marine algae (e.g., Chapman and Lindley, 1980; Dunton et al., 1982) which have a regular or seasonal growth pattern. The measurement of change in biomass is less suitable for the microalgae of the epipelic, episammic or epilithic associations, but can be effectively used with artificial substrates (e.g., Stockner and Armstrong, 1971). Oxygen, C-14 and DIC (dissolved inorganic carbon) methods are more applicable for instantaneous production rates of micro-algae (Round and Hickman, 1984; Turner et al., 1983). Descriptions of the first two basic methods are provided by Round and Hickman (1984). Schindler et al. (1973) and Turner et al. (1983) have given descirptions of the DIC method.

Of the above techniques, the oxygen method is quite suitable for phytobenthic production studies in remote locations, but suffers from problems of resolution at very low production levels which can be frequently encountered in the Arctic. In waters with high chemical oxygen demand such as in heavily polluted waters, the method will over estimate productivity and carbon uptake methods are preferable (Chrost and Sikorska, 1976). Unlike C-14 accumulation techniques, the oxygen method can provide estimates of respiration from the dark incubation samples, permittng estimates of gross photosynthesis by correcting the results of oxygen evolution in the light incubation samples. If the results of the oxygen method are to be comapred with results from C-14 accumulation methods, however, assumptions or knowledge of the photosynthetic quotient (P.Q.) assumptions must be made for converting the oxygen results to units of carbon, where:

$$\text{P.Q.} = \frac{O_2 \text{ evolved, moles}}{CO_2 \text{ uptake, moles}}$$

Schindler et al. (1973) have described techniques for simultaneous measurements of periphyton production in lakes with direct oxygen and DIC methods to allow calculation of P.Q. Unfortunately, the large amount of bicarbonate in marine waters makes direct measurement of changes in DIC impractical due to noise of DIC analyses (Turner et al., 1973). The value of P.Q. is generally assumed to be about 1.0 to 1.5 (Strickland, 1960). Thus, in most studies where conversions to carbon units are made there are untestable assumptions about the value of P.Q. (Schindler et al., 1973).

Based on comparisons with the DIC method in oligotrophic lakes, Turner (1981) found that results of the oxygen method were consistent and similar for photosynthesis-irradiance curves, photosynthetic depth profiles and daily intergrals of production (Turner et al., 1983). It was concluded that the oxygen method appears to be suitable for making production measurements of periphytic algae, although occasional calibration with carbon techniques would be required (Turner et al., 1983).

The C-14 accumulation method which is more sensitive than the oxygen method is generally thought to measure something between net and gross production, depending on light intensity and duration of the incubation period (Vollenweider, 1969). Wetzel (1965) has presented evidence that C-14 uptake measures net production for aquatic macrophytes. He also pointed out that heterogeneity or artificiality of substrate in C-14 measurements of periphyton production have made interpretation of early studies difficult. Recent attempts to overcome these problems for estimation of periphyton production are described later. Another problem with the C-14 techinque is self-absorption if plant materials must be radioassayed for C-14 uptake. Techniques for overcoming self-absorption are tedious and expensive (Schindler et al., 1973).

Control of variation in irradiance must be provided during all incubations for measurement of primary production, otherwise comparisons of results could be made mistakenly between incubations at saturated or suboptimal irradiances (e.g., Turner et al., 1983). In addition, production measurements in a variable and unpredictable light regime may lead to serious errors when attempting to extrapolate from shore in situ incubations to seasonal estimates of production (Fee, 1973, Schindler et al., 1973). In the laboratory, irradiance control can be provided by using an artificial light source of known intensities. In situ variations in irradiance can be monitored by an appropriate underwater irradiance meter (e.g., Li-Cor quantum meter with submersible photo-cell and flat cosine collector sensitive to light of 400 to 700 nm; Shearer, 1976; Turner et al., 1983). Surface irradiance can be monitored with a cosine-corrected flat plate collector (e.g., Li-Cor Li-1905) with output integration over 10 minute intervals (e.g., by a Campbell CR integrator; Turner et al., 1983).

For realistic estimates of primary production, techniques which minimize description of the studied algal community are recommended. The habitat or substrate of the algal assocation can be natural, such as sediment (epipelon) or rock surfaces, (periphyton) or it can be artificial, such as glass or smooth rock plates (periphyton) of known area colonised by the algal community (Turner et al., 1983). The use of colonised artificial substrates of known area overcomes the uncertainty of surface area associated with many natural substrates, but may result in a different species composition being sampled (e.g. Tippet, 1970).

Loeb (1981) and Turner et al. (1983) have described small incubation chambers (50 cm^2 and 200 cm^2 surface area, respectively) for C-14 or DIC in situ measurement of primary productivity of the epilithic periphyton. The devices for use on natural substrates consist of light and dark acrylic or Plexiglass chambers which can be sealed to the substrate by a neoprene skirt and led ring or sock. The chambers have rubber serum stopper sampling ports for servicing by diver. Turner et al. (1983) also described an incubation chamber for field laboratory incubation of a colonised 200 cm^2 plate of smooth granite. In the field laboratory technique, a gradient of irradiance of artificial light and many of the advantageous characteristics of phytoplankton incubator methods (Fee, 1973) could be employed.

For estimation of in situ production of microalgae in sediments by the oxygen method, it is desirable to measure changes in dissolved oxygen concentration over light and dark undisturbed sediment cores (Pomeroy, 1959; Pamatmat, 1968; Hargrave, 1969). Net production can be calculated as described by Hargrave (1969). Additional measurements of sediment core oxygen uptake (respiration) are needed to permit an estimate of gross production. The relative contribution of phytoplankton oxygen production and consumption in the water over the sediment should also be examined and corrected for if necessary. It is important to ensure that oxygen bubble formation does not occur during incubation as this will lead to a serious underestimation of production (Hunding and Hargrave, 1973). Shortening the incubation period during high levels of irradiance or reduciton of irradiance with light filters will lessen this problem.

The units used to express production results should give an indication of either the actual values measured or the validity of the results (Round and Hickman, 1971, 1984.) For instance, production of epilithic, episammic or epipelic algae should be expressed as mgC fixed da^{-1}m^{-2} or O_2 evolved h^{-1}m^{-2}. The time interval, time of day and irradiance levels of in situ incubations should be stated and the existence of a diurnal rhythm (e.g., Doty and Oguri, 1957) should be examined.

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6. POSITIONING

In any sampling program, some attention must be given to the determination of sample location. The required accuracy and precision of station positions will depend on the nature and requirements of the study. A baseline survey over a large area may not require highly accurate (or precise) positioning and errors of 5-10% of the distance between stations may be acceptable. Some site-specific surveys, however, where stations are to be monitored repeatedly or where changes over a small geographic scale are being investigated, accuracies of plus or minus several metres may be required. High precision positioning is also required for the deployment and recovery of underwater instrumentation. Some additional considerations in selection of a positioning system are:

1. Range - ideally one system should cover the entire study area, although this is not an absolute requirement. In general, systems that have a large range (Satnav, Omega) have reduced accuracy.

2. Accuracy and Precision - extremely accurate survey systems are available but their complexity and cost of operation are high. In planning a sampling program, it is necessary to decide in advance what standards of accuracy and precision are required.

3. Cost - there is a wide range in the costs of various positioning systems, both in terms of purchase/rental and operation. This is a major factor in selecting a practical system.

4. Size - in all arctic operations, large systems present a transportation problem.

5. Nature and Size of the Study Area - the physical nature of the study area may affect the selection of a suitable positioning system. For example, nearshore shipboard surveys in the Eastern Arctic may be carried out using radar as the primary positioning system, due to the presence of strong topographic features, whereas radar would not be suitable for a similar survey in the Beaufort Sea.

6. Availability of Positioning Systems - in some areas, shore-based aids may be in place on a temporary and permanent basis for other users. In some cases, the use of these can greatly reduce the costs and efforts required. For example, the Polar Continental Shelf Project maintains a Decca chain in some locations for specific projects. The Canadian Hydrographic Service maintains an ARGO system at certain times. Various industrial groups may also maintain similar systems (ARGO or SYLEDIS mainly). At some offshore sites, acoustic transponders may already be installed for positioning.

The essential features of a variety of positioning systems including some very sophisicated surveying systems are outlined in Table II-6.1. Systems have been grouped according to their approximate operating range. The systems have been rated as to approximate cost based on the following scale:

INEXPENSIVE	< \$5000 Cdn
MODERATELY EXPENSIVE	\$5000 - \$50,000 Cdn
VERY EXPENSIVE	> \$50,000 Cdn

Several of these systems are outdated (LORAC, RANA, TORAN). Several others require large and high powered shore stations that present a serious logistics problem, especially for arctic applications (e.g., Decca NOMAN, Decca MOBILE SURVEY, HIFIX). Brief discussions of the features of the remaining systems are given below.

a) LORAN-C:

LORAN-C is an excellent, low cost, high precision navigation system, but coverage is missing in most areas in the Canadian Arctic and only marginal in others. No plans are presently in place to extend LORAN-C coverage to the whole Canadian Arctic.

b) NNSS/TRANSIT/SATNAV:

SATNAV is an excellent, low cost navigation system that provides worldwide coverage. The accuracy is sufficient for a great many applications. The equipment is small and relatively low powered and requires little training to operate. A limitation is that continuous coverage is not provided, with time gaps of 15 - 25 minutes between successive fixes. Some moderately expensive receivers include inputs from other systems (log, gyrocompass, OMEGA and/or LORAN-C) providing a continuous display of position by integrating the satellite fixes and other data sources.

The SATNAV system on its own may be suitable for many programmes supplemented by more traditional dead reckoning between fixes. The systems are small enough to carry on tracked vehicles or small launches. No shore stations are required, and a single unit can be used in any location. This is definitely an important system to consider.

TABLE II-6.1
SUMMARY OF AVAILABLE POSITIONING SYSTEMS

A: LONG RANGE SYSTEMS (> 100 Km)

SYSTEM	TECHNIQUE	GENERAL COMMENTS	RANGE	ACCURACY	ADVANTAGES	DISADVANTAGES	REF.
LORAN-C	Time difference in arrival of pulses from master and secondary stations gives hyperbolic lines of positions. Transmissions at 100 Khz.	Low cost, high accuracy navigation system where suitable coverage exists. Reliable Loran-C signals not continuously available in Canadian Arctic waters. Excellent choice if coverage is extended to Canadian waters. An inexpensive system ($2,000 Cdn.)	up to 2800 km	0.2 - 0.5 km	Inexpensive, small easily operated receiver. Shore stations are permanent installations maintained by gov't. Positions have high precision. Receiver only required. Low cost ($2,000 - $5,000 Cdn.)	Only partial coverage available in Canadian Arctic waters.	3,9
LORAC	Phase difference between continuous waves from synchronous transmitters gives hyperbolic lines of position. (~ 2 Mhz)	This is an older group of systems commonly in use. Large, high powered shore stations are required. Very expensive system now outdated.	up to 550 km	5 - 120 m			4,2
RANA-H	Phase difference of continuous waves gives hyperbolic lines of position. (~ 2 Mhz)	An older navigation system. A minimum of 4 large, high powered shore stations are required. An expensive system, now outdated.	200 km (day) 160 km (night)	20 m			4,2
Decca NOMAD	Phase comparison of continuous waves gives hyperbolic lines of positions. (85 - 130 Khz)	Specialized survey equipment, large, bulky shore stations. An expensive system, now outdated.	460 km (day) 360 km (night)	15 - 30 m			4
Decca Mobile Survey	Phase comparison of continuous waves gives hyperbolic lines of position. (117 - 176 Khz)	Specialized survey equipment, large, bulky shore stations. An expensive system, now outdated.	270 km (day) 185 km (night)	10 - 60 m			4
TORAN	Phase comparison of continuous waves gives hyperbolic lines of positions. (1.6 - 2 Mhz)	Older survey equipped, large, bulky shore stations. An expensive system, now outdated.	250 km (day) 180 km (night)				4
NNSS/TRANSIT Satellite Navigator	Special receiver/processor derives position from data relayed from orbiting satellite.	A simple versatile navigation system for for all areas. Receiver/processor only required, low power consumption. Continuous positions not available due to small number of satellites. An inexpensive system, with receiver costs from $2,000 Cdn.	worldwide	40 - 500 m	Highly recommended system, several small systems presently available.	1-3 hours between fixes. Other navigation systems required between Satnav positions.	1,3
GPS/Navstar	Special receiver/processor derives position from data relayed from orbiting satellite.	This is the "new" satellite positioning system that will replace NNSS/Transit. Fully operational in 1988. Continuous positions will be available. Moderately expensive.	worldwide	100 m	Continuous positions available worldwide.	Not available presently.	1,3
OMEGA	Phase differences between continuous waves from synchronized transmitters. (10 Khz)	A system primarily designed for aircraft, not sufficiently accurate for most scientific requirements. Integrated systems (e.g., Satnav/Omega) have been successfully tested in Beaufort Sea. Moderately expensive.	worldwide	3.5 - 7 km	Worldwide coverage, moderately priced system. Permanent shore stations maintained by gov't. bodies. Integrated systems (e.g., Omega/Satnav) can provide more accurate positions.	Local and atmospheric effects can cause error. Signal paths over polar ice cap can be distorted.	4,1,9
ARGO	Phase differences between continuous waves from synchronized transmitters. (~ 2 Mhz)	A sophisticated portable, high accuracy surveying system. Requires installation of several shore stations. Very expensive system.	750 km (day) 250 - 415 km (night)	5 m	High accuracy over large area.	Shore stations have high current draw, may require T-E generators.	6,3
SYLEDIS	Propagation time of pulsed signals between master and shore stations gives hyperbolic lines of position, range/range mode also. (420 - 450 Mhz)	A sophisticated high accuracy surveying system. Multiple shore stations required. Computer processing required to maximize accuracy. Very expensive system.	~ 100 km	± 2 m	Good range, high accuracy.	Multiple shore stations required. Computer processing may be required to minimize accuracy.	7,3
Decca HIFIX/6	Phase comparison of continuous waves gives hyperbolic lines of positions. (1.6 - 2 Mhz)	High precision portable surveying system; system requires reinstallation of shore stations and antennas. Very expensive system.	up to 300 km	± 1 m	Good range, high accuracy.	Multiple shore stations required.	6,7,2
RAYDIST 76	Phase comparison of continuous waves from synchronous transmitters. Range/Range or hyperbolic modes of operation. (1.6 - 3.3 Mhz)	A high precision surveying system requiring installation of shore stations and antennas. For maximum ranges quoted a 30.5 m antenna is required at shore stations. In medium range mode shore stn. antenna 14.3 m long. Very expensive system.	450 km (day) 250 km (night) less range using med. range antennas	± 3 m	Good range, high accuracy.	Very large antenna are required for shore stations. High current draw for relay stations ashore.	

TABLE II-6.1
SUMMARY OF AVAILABLE POSITIONING SYSTEMS

B: MEDIUM RANGE SYSTEMS (30 - 100 Km)

SYSTEM	TECHNIQUE	GENERAL COMMENTS	RANGE	ACCURACY	ADVANTAGES	DISADVANTAGES	REF.
Tellurometer MRD1	Phase difference of transmitted and received signals yields distance (range/range). (3 Ghz)	A high precision surveying system.	100 m - 100 km		High precision, small size of components, readily portable		6
Decca MAXIRAN	Phase comparison of transmitted and received signals yields distance (range/range). (440 Mhz)	A high precision survey system. Very expensive system.	90 km	± 3 m			7
SYLEDIS	Arrival times of pulsed signals from master and slave station gives hyperbolic lines of position. Range/range mode also. (420 Mhz - 450 Mhz)	A sophisticated high accuracy survey system. Multiple shore station required. Very expensive system.	up to 100 km	± 2 m		Multiple shore stations required. One shore station is designated MASTER and is connected to a computer.	7
Decca MINIFIX	Phase comparison of continuous waves gives hyperbolic lines of position. (2 Mhz)	A high precision lightweight electronic positioning system.	70 km	± 2 m	High precision.		6
RADAN	Phase comparison of continuous wave from synchronized transmitters give hyperbolic lines of position. (2 Mhz)	A small easily portable electronic positioning system. Moderately expensive system.	70 km	10 m	Moderate precision and range.	Moderately high current draw at shore stations. Battery operation may not be practical.	6
RDF (radio direction finding)	Radio receiver equipped with direction sensitive antenna used to determine bearings to radio transmitter.	A sample system to use but relies on availability of suitable radio aids in appropriate locations to yield good fixes	50 - 90 km	highly variable depending on beacon orientation	Easy to use when radio beacons present.	Poor accuracy.	6,2
PULSED MICROWAVE RANGING SYSTEMS	Elapsed time between interrogation pulse and reply from shore station gives antenna distance (range/range).	A line of sight system with high accuracy. Very easy to use and install. Highly portable. Moderately expensive system.			High precision and easy to use.	Limited range. Very small, low powered shore units	6
a) MINIRANGER	5.4 - 5.6 Ghz		37 km	± 3 m			
b) TRISPONDER	9.3 GHz		30 km	± 3 m			6
c) RACAL	5.0 Ghz		30 km	± 3 m			3

TABLE II-6.1
SUMMARY OF AVAILABLE POSITIONING SYSTEMS

C: SHORT RANGE (LINE OF SIGHT) SYSTEMS (0 - 30 Km)

SYSTEM	TECHNIQUE	GENERAL COMMENTS	RANGE	ACCURACY	ADVANTAGES	DISADVANTAGES	REF.
Autotape	Phase comparison of transmitted and received signals yields distance (range/range). (3 GHz)	A high precision and portable system. Expensive.	L.O.S. (Line of sight)	± 0.3 m	High accuracy.	Very expensive; limited range.	7
Artemis III	Phase comparison of microwave signals with tracking antenna to determine bearing. (range/bearing) (9.2 Ghz)	A range/bearing microwave system. Only Only a single shore station required. Expensive.	L.O.S.	± 1.3 m	Single shore station only required.		7,6
Acoustic Transponders	Signal transit time from surface unit to transponder and response gives range.	Bottom mounted transducers must be deployed in known locations. Surface unit is located by slant ranges to bottom mounted units. Some processing required to get maximum accuracy. Systems vary from inexpensive to moderately expensive.	Up to 10 km for some systems	± 2 - 3%	Moderate price, surface equipment. Lightweight and easy to use.	Auxilary information required to get maximum accuracy (e.g., T, Sal vertical structure). Limited range. Requires secondary positioning system to determine position of transponders.	
Hydrodist MRB-3	Phase difference between transmitted received signals. Yields distance. (range/range) (1.3 Ghz)	An older surveying system.	30 km	± 2 m			2,4
RADAR	Time delay for reflected microwave signals to rotating antenna yields range and relative bearing to targets.	A standard shipboard navigation device. Utility highly dependent on presence of suitable targets. Moderately expensive.	L.O.S. 30 - 50 km	variable	Standard ships equipment, very easy to operate.	Requires presence of suitable targets. Not portable.	
Sextant	Measurement of horizontal angle between known targets.	A simple, portable hand held device capable of providing accurate measurements where suitable targets are available. Inexpensive.	L.O.S.	highly variable	A portable, hand-held device. Experienced operator can achieve high precision. Suitable for use in small launch.	Suitable targets required. Accuracy dependent on orientation of targets. Good visibility required	
Pelorus	Measurement of horizontal angles between known targets.	A simple device for measuring angles. Less precise than a sextant. Similar restrictions and capabilities. Inexpensive.	L.O.S.	variable	A simple portable device. Moderate accuracy possible. Suitable for use in small launch.	Suitable targets and visibility required. Accuracy dependent on orientation of targets.	
Theodolite	Measurement of horizontal angles between known targets.	A surveyor's instrument - equipped with a high powered telescope to aid in identifying and pointing at target. Suitable for on-ice work only.	L.O.S.	± 1 m	High precision, relatively easy to use.	Somewhat delicate instrument. Not suitable for use on water.	

c) GPS/NAVSTAR

Navstar (or GPS - Global Positional System) is the new satillite navigation system that is presently under development for the U.S. Military. Civilian users will have access to degraded position data with an accuracy of approximately 100 m. In addition, continuous position reports will be available when the system is fully operational in 1988. This system will eventually replace NNSS/TRANSIT/SATNAV. This system is widely regarded as the navigation and positioning system of the future.

d) ARGO

ARGO is a very sophisticated, highly accurate survey system that is being used by the Canadian Hydrographic Service and various companies in the Arctic for precision positioning. This is a very expensive system to purchase, and shore stations must be established and maintained. A single set of shore stations will, however, support multiple users in the range/range mode.

e) SYLEDIS

SYLEDIS is also a highly sophisticated, highly accurate survey system. This system has been used by oil companies for exploration work in the Canadian Arctic. As with ARGO, it is an expensive system, but multiple users can be supported.

f) PULSED MICROWAVE SYSTEMS (e.g., Motorola Miniranger, Del Norte Trisponder, Racal Microfi)

These are small, highly portable high precision positioning systems used in line of sight application. The range that can be achieved is dependent on the height of the transceiver units. The systems are easy to install and operate, although maintaining suitable batery power at remote stations in very cold weather may present some problems.

g) RADAR

Standard shipboard radar can be used to provide good positions, provided suitable targets are available. This is highly dependent on the location and size of the study area. For nearshore, ship-based surveys in areas with suitable targets, this is a reliable and versatile system.

h) SEXTANT/PELORUS/THEODOLITE

All three devices can be used to measure horizontal angles between suitable targets. Obviously, good visibility is a requirement, which presents a severe limitation for many Arctic operations. In addition, the study area must possess appropriate targets from which to measure. For very nearshore surveys it may be appropriate to erect mains or masts as targets for the duration of the sampling program. All three instruments are light weight and easily used. The sextant and pelorus are suitable for use on a small launch, whereas the theodolite requires a stable base (on ice, for example)

A summary of suggested positioning systems for different applications is given in Table II-6.2.

TABLE II-6.2

SUGGESTED POSITIONING SYSTEMS FOR VARIOUS APPLICATIONS

SAMPLING PLATFORM	SURVEY TYPE	ACCURACY REQUIRED	SUGGESTED SYSTEMS
1. Shipboard	Wide area survey	100-500 m	SATNAV[3] (offshore) SATNAV[3] plus RADAR (nearshore)
		1-10 m	ARGO, SYLEDIS
	Nearshore	10-100 m	RADAR [1]
		1-10 m	MINIRANGER/TRISPONDER/ MICROFIX
2. Small Launch	Nearshore	10-100 m	SEXTANT, PELORUS [1,2]
		1-10 m	MINIRANGER/MICROFIX/ TRISPONDER
3. Ice	Wide area survey	100-500 m	SATNAV
	Small area	10-100 m	SEXTANT, PELORUS, THEODOLITE[1,2]
	Small area	1-10 m	MINIRANGER/MICROFIX/ TRISPONDER

1. suitable targets required
2. good visibility required
3. continuous positions not available; dead reckoning required between fixes.

6.1 References for Table II-6.1

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5. Jeffery, C.B., 1970. Positioning Systems for Great Lakes Surveys. An unpublished report by Geospace Engineering Co. to the Canada Centre for Inland Waters, Burlington, Ontario.

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7. Marks, A.J., 1978. An Evaluation of Marine Microwave Precision Systems. Institute of Oceanographic Sciences, unpublished Internal Report No. 70.

8. U.S. Department of Transport, 1980. LORAN-C User Handbook. Designated COMDTINST M16562.3. 82 pp.

9. Mortimer, A. and P. Milner, 1980. LORAN-C and OMEGA Navigation System Tests in the Beaufort Sea. Institute of Ocean Sciences (Sidney, B.C.). Pacific Marine Science Report 80-4. 84 pp.

7. SAMPLING PLATFORMS

Despite the fact that arctic marine areas are ice covered for at least 8 months of the year, most sediment sampling has been carried out from ships during the open water season. This has been a matter of convenience and in many instances the only practical means. Sampling from the ice is bound to increase in importance, however, as information requirements increase and as equipment portability is refined.

7.1 Sampling from the Ice

Sampling from the ice is practical only when the ice is relatively stable. In nearshore areas this means until the ice has achieved sufficient thickness, usually a month or so after freeze-up until a few weeks (or less) prior to break-up. Further offshore and in many coastal regions multi-year ice may be present all year. Sampling from the ice has some advantages, most notably a stable platform and large surface area, as well as some obstacles not encountered in more traditional shipboard sampling, including access to the water, shelter and transport.

There are no standard procedures or equipment for sampling from the ice. Requirements and equipment vary with the application, transport restrictions, time of year, personal preferences and available resources. There are, however, several factors that should be considered when planning, namely:

1. site selection;
2. drilling holes in the ice;
3. positioning;
4. shelter and safety requirements;
5. transport;
6. equipment, power, winch and retrieval requirements; and
7. a practical sampling device.

The factors in sampler selection will be a function of goals, parameters to be analysed and portability. These topics have been reviewed in Sections II-2.2 and II-2.3. There is a practical limit to the size of hole that can be readily made in the ice (section 7.1.2). In general, the most compact sampler (smallest cross-sectional dimensions) capable of satisfying the objectives should be used. The other factors are discussed briefly below.

7.1.1 Site Selection

In first year, land fast ice, site location is usually not a problem. The ice is relatively flat and a uniform thickness. In transition zones or multi-year ice however, site selection is more difficult. This ice can be very uneven and broken. An area of flat first year ice is preferred as this will have a minimum thickness and provide a flat landing area for aircraft and equipment. Where the ice is ridged or tilted, ice thickness may be much greater than anticipated. This will require more time to make a hole in the ice or perhaps make it impossible with the equipment available. Multi-year ice will be thicker (2 - 10m) and harder in general. Sampling on transition or multi-year ice will require considerable flexibility in site location. The number of sites and their location will be dictated by local conditions and cannot be planned in detail in advance (Section 2.1.2).

7.1.2 Making a Hole in the Ice.

Once a location has been selected, a hole of sufficient size for the sampling gear is required. Small corers without stabilizer fins may require a hole of 25 cm diameter or less. Small grab samplers such as the Ekman will require a hole at least 30 cm in diameter. Holes of more than 80 cm will be required for larger grabs. It is often advantageous to drill a small diameter hole first to determine ice thickness. This can save a lot of time if the ice proves to be too thick for the equipment available.

There are a number of ways of making a hole in the ice, depending on the ice thickness, size of hole required and the equipment available. In the early winter when the ice is relatively thin, a sharp ice chisel is the simplest means available. The most common method, however, is to drill a hole using commercially available ice augers and power drives. The augers can be driven by gasoline powered engines, by electric motors, by a hydraulic drive or by hand. It is generally not practical to use a hand auger for holes greater than about 10 - 15 cm in diameter in thick ice, which is too small even for gravity corers. With a power unit, either gasoline driven or electric, up to 45 cm holes can be drilled, though larger sizes (> 25 cm) require additional horsepower in the drive unit and are usually heavy. These units can penetrate 2 m of ice in about 10-20 minutes. If a large track vehicle is available, a vehicle mounted drill can be used driven by vehicle power or hydraulics and larger diameter augers used (up to 60 cm).

Portable gasoline powered engine drives have the advantage of portability, wide spread commercial availability, and low cost (typically under $1,000). The units are heavy however, and can be difficult to start in very cold weather. Two people will be required to drill smaller diameter holes (to 25 cm), with an extra person required for larger diameter units in thick ice.

Electric drills are also readily available. However, they require a power source (generator) which may present problems. Large drills with up to 1 hp motors are required.

For large size holes, chain saws with long extensions have been used. This is a laborious, time consuming (usually several hours) procedure which is not recommended for routine work. However, for a limited number of sample sites, this is an effective means of making a large hole. For diving, this may be the only practical method for making a large enough hole.

Perhaps the best and certainly most versatile method available is the hot water drill. Hot water drills are not available commercially but can be built from readily available components and materials. Verrall and Baade (1982) developed a unit for drilling holes in ice shelves along Ellesmere Island. They were able to put a 23 cm diameter hole through 50 m of shelf ice at a rate of 11 m/hour. The system consists of a pump, water heater, heat exchanger, melt tank, control valves, drilling head and hosing to deliver water down the hole and return it. (A schematic diagram is shown in Figure II-7.1). The water heater consumed turbo fuel at the rate of 13.3 L/hour. The drilling head used was a 1.5 m length of 2.5 cm steel pipe. Hole diameter was controlled by a conical weight which also acts as a pick-up for returning cooled water to the surface.

The Frozen Sea Research Group (FSRG) at the Institute of Ocean Sciences, Pat Bay, B.C., have also developed a hot water drill for use in arctic applications. The FSRG unit works on the same principle except that they utilize an oil burner (3 USGPH fuel consumption) and have produced a helicopter and Twin-Otter fixed wing aircraft portable design. FSRG also utilizes a melting ring rather than a single jet outlet. Two perforated rings are used in tandem, one for the outlet of hot water and one for collecting cooled return water. This system allows a great deal of flexibility as rings of various diameters can be easily fabricated depending on the application. Very large holes can be made by drilling several holes side by side. Using a 91.5 cm diameter ring, the FSRG unit can penetrate 2 m of ice in about 35 minutes. The ice

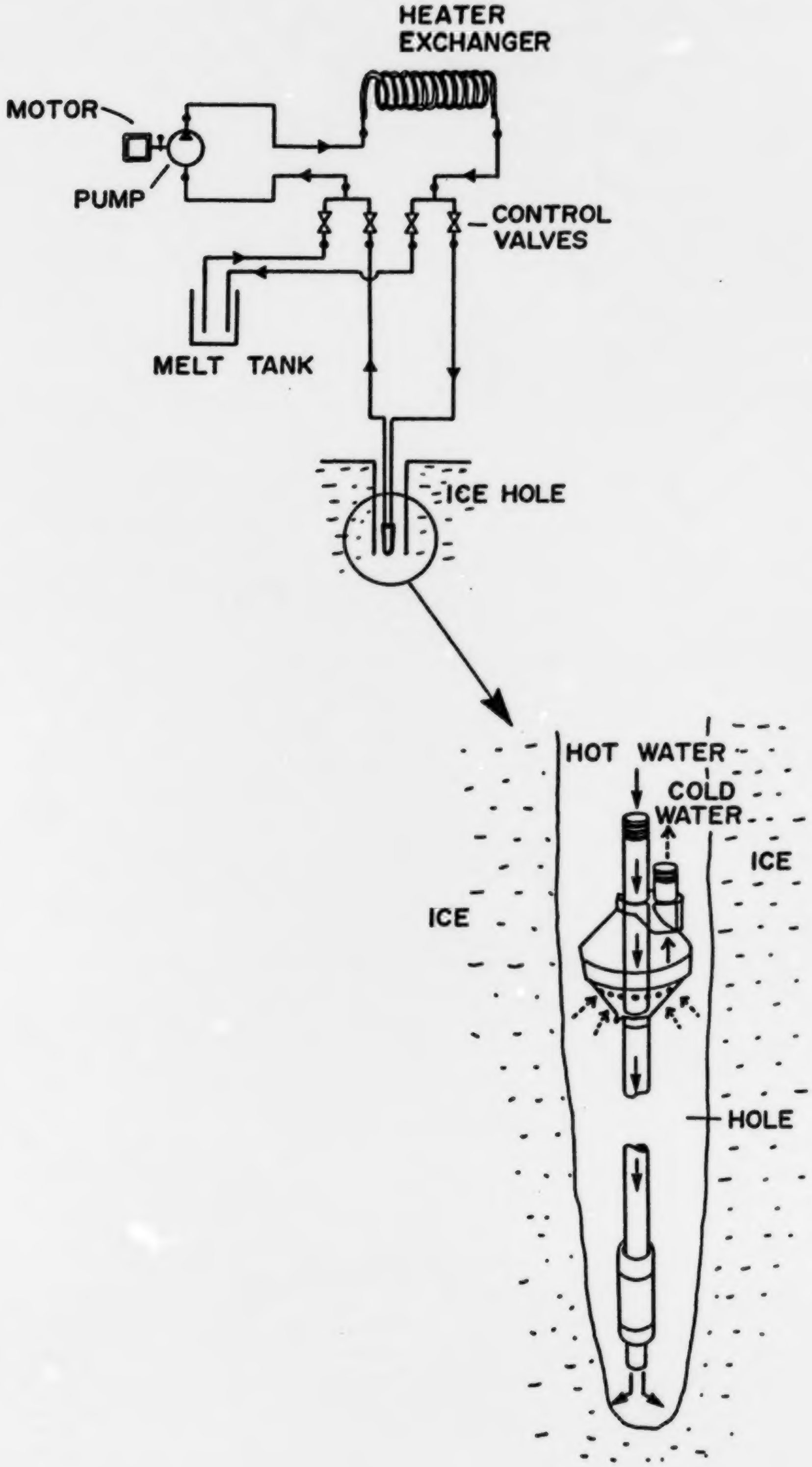
HEATER
EXCHANGER
MOTOR
PUMP
CONTROL
VALVES
MELT TANK
ICE HOLE
HOT WATER
COLD
WATER
ICE
ICE
HOLE

Figure II-7.1. Schematic diagram of a hot water drill and head (from Verall and Baade, 1982).

block that is produced can either be pushed down under the ice or removed. The ice hole produced by either method has the additional advantage of being free of slush and ice chips produced in other drilling methods.

The chief disadvantages of this method are the requirements for a generator to run the pump and heater and the lack of a commercially available system.

7.1.3 Positioning

This topic has been reviewed in Section II-6. An advantage to an ice platform is that it is either immobile or is drifting very slowly. For the purposes of sediment samples, once a position is fixed, it is not necessary to correct for drift while sampling.

7.1.4 Shelter and Safety

Except for ideal conditions in late spring or early summer when air temperatures may be above freezing, some sort of shelter will be required while on the ice. The type of shelter required will depend on the length of time spent on location, weather conditions, transport limitations and sampling gear. The simplest shelter is a windbreak but usually a heated area is needed both for personnel and equipment. This may be the transport vehicle (all terrain vehicle, snow-trak, airplane or helicopter); a portable insulated shelter or tent (Parcoll, Hansen or Norseman tent); a trailer that is either helicopter portable (Erickson et al., 1983) or mounted on skis (FSRG, Pat Bay); or a snow hut/igloo. Depending on clearance requirements and size of ice hole and sampler, sampling may be carried out within the shelter (Erickson et al., 1983; Herlinveaux and de Lange Boom, 1975).

Sampling on the ice demands that the sampling party must be totally self-sufficient. Equipment requirements should reflect this and allow for emergency situations (communication equipment, survival gear, food, fuel, first aid equipment). Sampling on the ice also requires that the sampling party be aware of the dangers of an encounter with polar bears should the party be on the ice for a long time. Some sort of watch should be maintained and a rifle of suitable power (as well as someone with the knowledge of how to use it) must be readily available. Polar bear deterence strategies may be obtained from the Government of Northwest Territories (GNWT), Department of Renewable Resources - Wildlife Service, Habitat Management Section.

7.1.5 Transport

Depending on the distance to be travelled and the amount of equipment required, a number of possible means of getting to a location are available. For short distances close to a community or camp, a snow vehicle (Snowmobile, Bomardier, ATV or Cat) with a sled for equipment is suitable. As distances increase, all transport will eventually rely on aircraft, either helicopters or fixed wing. Helicopters are more versatile but also more expensive to charter and not as readily available. Choice will depend on the area to be sampled, amount of equipment and distance to be travelled. Fixed wing aircraft are required when distances are great (generally more than 100 km). Helicopters have advantages on shorter flights in terms of flexibility (landing), in site selection and in handling bulky items (these can be carried in a sling under the aircraft). People cannot be transported simultaneously when equipment is carried in a sling. Aircraft cannot be used when visibility is poor, as a result of weather conditions, or darkness, so that long delays may result at certain times of the year.

7.1.6 Power, Winch and Lifting Requirements

With the exception of the small Birge-Eckman dredge, almost all sampling will require a winch and davit (or boom) to lower and retrieve the sampler (see section II-2; Table II-7.1). Lifting requirements and the size of winch and power required as much as the sampler dimensions will dictate the type of sampler that can be used. All equipment must also be portable enough for aircraft transport. Winches can be gasoline powered, electric or hydraulic. Drive units for ice augers can often be adapted to drive a winch. Tripods or quadrapods have been used to support the block above the ice hole. Winches can be supported directly on the frame. (Thomas et al., 1984; FSRG.)

There is no commercially available equipment "ready to go". Equipment components must be designed and mated to satisfy the sampling needs. Experience from others who have sampled from the ice will be helpful. Ultimately, however, investigators must adopt methods to suit their specific requirements and must thoroughly test out equipment and procedures before finalizing a sampling plan.

7.2 Sampling From Vessels

Vessel requirements for sediment sampling in arctic waters will be the same as for other marine areas with the exception that, in some instances, vessels with an ice-strengthened hull will be required. The other major constraint is the general lack of suitable vessls in the Arctic so that choices will be limited and costs will be higher. Charter costs for a 40' fishing vessel with operator when available will be in the range of $2,000/day plus fuel. Larger vessels must usually be brought in from southern areas at great expense. In more remote areas, the only boats available may be 15' - 20' fishing boats or canoes, or inflatable boats flown in. Obviously this will restrict the type of samplers that can be used.

The following factors should be considered when formulating a sampling plan for sampling from a boat:

1) lifting capability: winches, line (strength, material and length), blocks, booms, cranes, A-Frames, davits; (see Section 2.3 for approximate lifting requirements; see U.S. Naval Oceanographic Office, 1968; Stirn, 1981, or Holme & McIntryre, 1981, for details). Table II-7-1 outlines typical applications for various types of winches and wire rope;
2) clearance over the railing with available lift capacity (function of sampler dimensions);
3) open work area for sampler manipulations, storage, etc.[a] (this will depend on the type of sampler, the amount of equipment and number of people required to launch and recover sampling gear);
4) laboratory space (for sub-sampling, sample work-up);[a]
5) storage facility (freezer and refridgerated space);
6) navigation equipment (see Section II-6);
7) accommodation;
8) range (fuel capacity, rate of consumption, speed);
9) anchoring capability;
10) availability of electrical power;
11) availability of running water (benthic sampling);
12) overall dimensions, sea-worthiness, ice rating; and
13) communications - types of radios, frequencies available and range.

[a] Note: Ships present very "dirty" environments for sampling for trace metals or trace organics. Special care must be taken to prevent contamination.

TABLE II-7.1

TYPICAL APPLICATIONS FOR VARIOUS WINCHES AND WIRE ROPE LINES

DUTY	APPLICATION	WIRE ROPE SIZE (mm)	LENGTH OF LINE (m)	LOAD (kg)	SPEED (m/min)	MOTOR (H.P.)
Light	smallest grabs	2.4 - 3.2	400 - 800	50	300	3 - 5
Medium	all grabs, light to medium weight cylindrical and box corers	4 - 5	up to 5000	250 - 500	100 - 300	15 - 20
Heavy	piston corers, large box corers and gravity corers	9.5 - 12.5	to 12,000	14,000 - 3,600	40 - 120	30 - 150

7.3 Literature Cited

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8. TRANSPORT OF SAMPLES

Sample transport from arctic regions is the portion of the analytical chain linking the field and laboratory portions of the study. This is a critical step, as the investigator loses control of the samples. Precautions must be taken to ensure the integrity of samples during transit and their timely delivery to the laboratory.

In the Arctic, all transport will ultimately be by air, perhaps with several transfers. Prior to the trip, air carriers, routing, schedules and cargo restrictions must be known so that transport can be planned ahead. Few northern coastal communities have daily flights to southern Canada and all will have weight/size restrictions for cargo. A list of air carriers, licence restrictions and addresses for major arctic communities is given in Appendix C. Some points to be considered when planning sample transport are:

1) cargo capacity of the aircraft (limiting dimensions for a single item and weight restrictions);

2) schedules: general availability of space, rates, priority freight service;

3) terminal facilities: are there facilities to keep samples cool, frozen, or to keep them from freezing in winter at transfer points?

4) restricted items: several items such as dry ice, concentrated acids, formalin, batteries (wet), gasoline cans, gasoline motors may not be accepted for carriage on passenger carrying aircraft. All restricted items require proper documentation regardless of aircraft and packaging. Failure to do this can cause delays or result in loss of necessary items (as well as potentially severe penalties). IATA (International Air Transport Association) publishes a listing of all restricted items, along with instructions for proper packaging, labelling and documentation. Permissable quantities are also indicated. The most recent IATA regulations should be consulted when planning a field trip (IATA regulations may be obtained from Traffic Services, 2000 Peel Street, Montreal, Quebec, H3A 2R4);

5) packaging: wooden, metal or hard plastic should be used when possible; some plastics become very brittle at low temperatures. All plastic containers should be tested before being used to transport materials in winter months. Cardboard boxes should not be used;

6) labelling: all items to be shipped need a number, the contents of each box should be recorded; labels should clearly show destination, special handling instructions and phone numbers to call on arrival; restricted items need proper documentation (item 4);

7) cost: charter rates, freight rates can be a major factor in the overall budget. In planning a sampling program, allowance should be made for the extra weight of samples on return trips;

8) timing: depending on the amount of equipment to be transported, it may be necessary to ship equipment ahead. When personnel follow, they can then ensure that all equipment reaches its destination on time.

The key to successful transport is good planning as well as the necessary flexibility to deal with forced delays due to weather.

9. LEGAL SAMPLES

Whenever it is known or suspected that samples will be required for litigation, additional precautions are required in sampling, handling, storage, transport and analytical procedures.

Dick and Pant (1978) summarized three fundamental objectives when dealing with legal samples for oil spills which can also be applied to sediment. These are:

1) the preservation of sample integrity so that the sample composition is the same as at the time of sampling;

2) the proof of continuity of the sample from sampling through to final documentation (chain of custody); and

3) the use of analytical techniques capable of providing positive identification of the pollutant.

Maintaining sample integrity is a function of the sampling and storage procedures and is the goal of all sampling programs. Available analytical techniques are discussed in Section III. The proof of continuity of the sample is to assure and document that the sample (s) is in the physical possession of a specified individual or in a secured place and could not have been damaged or altered (Katz and Jenniss, 1983). This requires special procedures not usually followed in routine applications. There are apparently no well defined rules to ensure continuity. Dick and Pant (1978) in their review outlined various methods in current use in Canada and the United States.

In preparation for field sampling, a sufficient number of appropriate containers should be assembled, with etched or permanently marked identifying numbers on the containers as well as on matching lids. The containers should then be properly cleaned and prepared, as specified for the particular analyte, by one lab person only. To reduce the possibility of contamination, the sample containers, lids and labels should be sealed individually into polyethylene bags. The containers are then packed into a locking sample box in readiness for field sampling. The box is marked "legal sample bottles", accompanied by the date and a signature of the person who prepared them, and then locked. At this point, the sample box key is given to the person in charge of sampling and the sample box is transferred by this person to the sampling site.

At the sampling site, field personnel are required to keep a field notebook of all pertinent information. For each sample taken the container number, date sample taken, time sample taken, source of sample (with reference to an area diagram), preservative, if used, and physical characteristics, where applicable, should be recorded in the field notebook as well as on the label supplied with each sample container. The field sampler's name and signature, as well as that of an emergency or investigating officer or witness should be recorded on each sample label (if possible). The label should then be securely fastened to the sample container and taped over with transparent tape to preserve the information. The container number is cross checked in the field notebook with the information on the label.

After being filled with the sample, each container is sealed and should be cross-taped and marked in such a manner as to prevent contamination and leakage, and to indicate if the container has been tampered with.

At least two sample containers should be left empty as a control, to prove that the containers were not contaminated before sampling. All possible suspected sources relative to each sampling site should be sampled, with a minimum of two samples from each location.

The sample box, with all the sample containers, is transported with the crew to each sampling location. Samples and the sample box itself, are not left unattended. After completion of sampling, the sample box is locked. The key should be kept on the field investigator's person at all times, and the sample box is clearly marked as to its contents. Samples must be in the field investigator's actual physical possession; or in an area where they can be kept under surveillance by an authorized person; or in a limited-key-access locked refrigerator or storage area, where they cannot be tampered with until such time that they can be brought to or sent to the appropriate laboratory for analysis.

Samples can be sent to the lab for analysis by registered mail, air freight or air express. Any transport documents associated with shipping, such as a hazardous material shipping form, are retained for the legal file. All wrappings and sample containing boxes are all similarly identified. A covering letter is prepared with similar identification and one copy is sent separately to the lab involved while another copy accompanies the samples. A memorandum describing the samples and the type of analysis required is included in the sample box. A key to the sample box is sent in a separate envelope and not attached to the box. The box lock is secured and taped-over. An added precaution that has been suggested is a photograph showing the condition of the box.

Hand delivery of the samples to the laboratory by the field personnel is preferred. The associated identifying documentation, memorandum of sample description and required analysis is also hand delivered. The laboratory person receiving the delivery and the person making the delivery sign a transmittal slip with copies being kept by both parties. The name of the person making the delivery is noted in the case file. A note of the transmittal is recorded in the laboratory record book or file and any additional receiving data is then noted, such as evidence of tampering. All packaging and wrappings are dated and initialled by the analyst and then retained on file.

Deliveries by registered mail or courier should be received by the analyst assigned the task of carrying out the analyses for the samples in question. Upon receiving the sample box, the analyst should take a photograph of it, to compare with a photo taken in the field. All registration slips or waybills should be kept with the box, and any wrappings initialled and retained.

With the arrival of the samples in the lab, a case file should be opened and case numbers assigned to each sample. A continuity sheet is prepared and the samples examined in conjunction with the memorandum requesting analysis.

All samples are marked by the analyst with his/her initials, the case number, the sample number and the date of receipt. All memoranda and associated documents are similarly marked, and the condition of the samples noted in the laboratory notebook.

One analyst should carry out all of the analyses and keep all samples, process solutions, etc., under lock and limited access key when not under direct surveillance. Notes are taken of all aspects of the analysis. All such notes, graphs, charts, and rough drafts of final reports should also be kept under lock and limited access key during the analytical process and when not in direct use.

Subsequent to laboratory analysis, the analyst should ensure retention of sufficient post-analysis sample for defence or rechecking purposes. Where adequate storage facilities exist, the post-analysis samples are retained under lock and limited-key-access until prosecution is completed or until the case is dropped.

If adequate locking storage space is not available at the lab or cannot be arranged, the analyst should return the samples with the final report to the originating field group. The samples are transferred either by hand, registered mail, air express, air freight or courier. If transferred by hand, a signature is obtained on the laboratory continuity sheet from the person receiving the shipment. If shipped by registered mail, express, etc., all documents involved in making the shipment and transmittal are retained in the case file. The receiver of the shipment signs the continuity sheet and holds the samples in a locked, limited access key area until the legal process is completed or the case dropped.

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SECTION III: ANALYTICAL METHODS

1. INTRODUCTION

Once a sample has been collected, it is generally necessary to store and return it to the laboratory for analysis of the parameter(s) of interest. Although the analyst can exercise far more control in the laboratory, practical constraints will still be important in method selection. Choice of analytical method must be made in the context of the goals, use of data, cost and the limitations imposed by sampling. It would be a waste of effort, for instance, to apply an expensive analytical procedure providing optimum precision to a poorly collected sample. Youden has stated that there is little merit in reducing the analytical uncertainty to much less than one third of the sampling uncertainty (cited in Kratochvil and Taylor, 1981). Measurement procedures must therefore be viewed in the overall context of the study and be revised if necessary in light of the actual performance of the sampling methods. Regardless of the methods chosen, however, certain elements of quality assurance must be built in to the analysis to allow quality statements to be made about the resultant data and certain minimum levels of reporting adhered to (Keith et al., 1983; Macdonald and Nelson, 1984).

1.1 Evaluation Criteria

This section presents a review of laboratory analytical methods in use for the measurement of specified parameters in sediments and biota. The methods have been reviewed where possible in terms of their performance characteristics and how these relate to known properties of arctic marine sediments and benthic organisms. Some of the performance characteristics and factors that have been considered are:

1. Sources of error - precision and accuracy.
2. Potential interferences.
3. Limit of detection and quantification (LOD, LOQ).
4. Available reference materials.
5. Blanks.
6. Time requirements and cost.

It is useful before beginning to elaborate on some of the above terms as there is, in many instances, ambiguity in the literature as to their meaning.

1.1.1 Accuracy and Precision

Accuracy and precision are terms commonly used to describe the performance characteristics of a method. **Precision** has been defined as the degree of mutual agreement among individual measurements made under prescribed conditions with a "single test procedure" (ASTM, 1977). Precision (or more properly imprecision) can be measured by the spread of the data and is a result of **random** errors.

Accuracy can be defined as the degree of agreement of measurements with the true value or accepted reference value (ASTM, 1977).

Although all methods strive for a high degree of accuracy and precision, there is no general agreement on a means of calculating and expressing these concepts. As a result, it is necessary when using these terms to:

1. describe how the computation was made; and

2. be explicit as to how the data were obtained (Youden and Steiner, 1975).

The most useful definition of accuracy when applied to the analysis of pollutants in sediments or biota will be one based on individual measurements rather than the average of a number of individual measurements (Kirchmer, 1983), since this will more closely reflect the way measurements are made. The former method defines the error, E, of an analytical result, R, as $E = R - t$, where t is the true value. The latter approach would define the error as $E = \bar{R} - t$, where $\bar{R}$ is the mean of a number of individual measurements. In both cases, as E decreases, accuracy improves.

Defining E in terms of individual measurements means that accuracy will comprise both random and systematic errors. Accuracy statements using this definition therefore describe the degree of precision and bias (the two components of accuracy). Defining E in terms of the mean of several measurements means that accuracy is a measure of the systematic error or bias only.

Simple statements concerning accuracy of a method can therefore be quite misleading if the means of calculating accuracy are not stated. In sediment and biota analyses, true values are seldom if ever known. Accuracy statements, if given, are therefore based on the performance of the method with recoveries of reference materials, standards or reference methods.

Because true values are not known, the precision of sediment and biota methods, often defined as the percent relative standard deviation of a series of measurements of the same sample, are frequently used in describing the capability of a method. It is possible for a method to be precise but not accurate however, so that reference material analysis and collaborate testing are crucial when evaluating results from different laboratories using different procedures. Unfortunately, this type of information is generally not available. As accuracy is one of the most important performance characteristics when assessing methods, the lack of good accuracy statements severely limits the comparisons that can be made.

1.1.2 Limit of Detection (LOD) and Limit of Quantification (LOQ)

These concepts have been reviewed in several recent reports (Keith et al., 1983; Long and Winefordner, 1983; Kirchmer, 1983; Glaser et al., 1981). Detection limit is a very commonly used term in determining the sensitivity of a method or instrument but its exact meaning is rarely defined. The definition recommended by IUPAC and the ACS Committee on Environmental Improvement (Keith et al., 1983) is "the lowest concentration level that can be determined to be statistically different from a blank". Procedurally, there are a number of ways of expressing LOD based on what is considered "statistically different". Traditionally, LOD has been expressed in terms of instrumentation as the smallest signal above background noise that an instrument can reliably detect - generally 2 x background. This can be properly termed the instrument detection limit (IDL) and although useful for comparing instrument capability, it is not a measure of the LOD of a method.

IUPAC defines LOD as $3\sigma_B$ where σ_B is the random error associated with the blank. In practice σ_B is estimated by the standard deviation of the blank (s_B). (s_B approaches σ_B as μ becomes large; when μ is small, s_B may seriously underestimate σ_B). This means of expressing LOD is based on an assumed normal distribution. A measured value will be greater than the blank at the 99% confidence level for a normal distribution when the measured value is three times the σ_B. This concept is obviously not applicable when the blank signal is below the IDL. In these cases, the IDL could be used.

An alternate means of calculating an LOD has been given by Long and

Winefordner (1983). This method takes into account the calibration error and is calculated using the propogation of errors method. Either method is acceptable (as are others). The important factor is that when LOD data are presented, the method used for their calculation needs to be stated.

The method detection limit (MDL) is defined as "the minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero" (cited in Glaser et al., 1981). This definition differs from the IUPAC one only that it is not defined in terms of the blank. Methods of determining MDL are given in Glaser et al. (1981). LOD will be numerically equivalent to MDL as defined by IUPAC as the blank approaches zero.

The limit of quantification is a far less commonly used concept. It is meant to indicate the level above which quantitative analysis can be made. It is therefore an equally important term for describing method performance. Basically, there are three analytical regions:

1. a region of unreliable detection below the LOD;
2. a region of qualitative analysis where the analyte is detected but the uncertainty is high; and
3. a region of detection where quantitative analysis can be made with a high degree of confidence.

Again, what constitutes a high degree of confidence will be variable. The ACS Committee on Environmental Improvement has recommended that LOQ equal $10s_B$ as this would correspond to an uncertainity of ±30% at the 99% confidence level for a normal distribution. Currie (1968) used a value of $14.1\ s_B$. The value of $10s_B$ would appear to be a minimum. The ACS Committee on Environmental Improvement have recommended that quantitative interpretation, decision making and regulatory action should be limited to data which is at or above the LOQ (Keith et al., 1983).

Limits of detection in the literature are most frequently given in terms of the IDL. Standard deviations of the blank are rarely given so that calculation of LOD and LOQ using criteria above is not possible. Comparison of the sensitivity of methods, reported in existing manuals and current literature is therefore usually only possible on an approximate basis.

1.2 Quality Assurance in Analytical Methods

Quality assurance is a term used to describe a program designed to reduce measurement errors and to assure that the results have a high probability of being of acceptable quality. A quality assurance program embodies two concepts: quality control, the mechanism established to control errors; and quality assessment, the system used to verify that control is working (Taylor, 1981). The topic of quality assurance has been discussed in detail in several recent reviews (Taylor, 1981; Keith et al., 1983; and Kirchmer, 1983). The goal of a quality assurance program is to bring a measurement system into a state of statistical control and subsequently maintain it there. Statistical control implies that procedural errors have been reduced to acceptable levels and have been characterized statistically.

Quality has a subjective definition. Acceptable data in one study may be found lacking in another. There are no set rules that one can apply. It is only possible to outline some of the elements that go into quality control and assessment. The actual methods used will be dependent on the intended use of the data, the laboratory and demands of the situation.

General elements of quality assurance have been summarized as (after Inhorn, cited in ACS Committee, 1980).

1. Maintenance of skilled personnel, written and validated methods, and properly constructed, equipped, and maintained laboratory facilities.
2. Provision of representative samples and controls.
3. Use of high-quality glassware, solvents, and other testing materials.
4. Calibration, adjustment, and maintenance of equipment.
5. Use of control samples and standard samples, with proper records.
6. Directly observing the performance of certain critical tests.
7. Review and critique of results.
8. Tests of internal and external proficiency testing.
9. Use of replicate samples.
10. Comparison of replicate results with other laboratories.
11. Response to user complaints.
12. The monitoring of results.
13. Correction of departures from standards of quality.

An important first step in the use of any analytical procedure is validation of the method. This means establishing key performance characteristics (accuracy, precision, detection limit, blank) and bringing the method into a state of statistical control (Taylor, 1983). Some specific quality control and assessment procedures used in marine sediment and biota analyses are:

1. collaborative testing programs;
2. analysis of reference sediments and tissue
3. analyses of blind replicates and control samples, and
4. use of control charts for assessing the quality of the results. (Keith et al., 1983; ASTM, 1976; Kirchmer, 1983).

For many types of analyses, reference materials are not available and quality assurance programs must emphasize components of the measurement system such as blanks, replicate analyses, spike recoveries and collaborative testing.

Much of the available literature on analytical methods and their application does not contain a description of quality control procedures or results. Without this information, it is difficult to assess the quality of the data or to make comparisons between different methods and data sets.

1.3 Documentation and Reporting

Means of presenting data will vary with the intended use and the type of measurement. Individual analyses are discussed in the following sections and a review of the requirements of reporting for legal sampling is given in Section II-9. There are, however, general guidelines for reporting data which are applicable regardless of the objective or the analyte. The general requirements for documentation and data reporting have been outlined recently by Keith et al. (1983) and are applicable to all environmental sampling and analyses. The major recommendations are reproduced below:

1) Documentation of analytical measurements should provide information sufficient to support all claims made for all the results. Documentation requires all information necessary to (a) trace the sample from the field to the final results, b) describe the methodology used, c) describe the confirmatory evidence, d) support statements about detectability, e) describe

the Quality Assurance program and demonstrate adherence to it, and f) support confidence statements for the data. Methods of determining detection limits, accuracy and precision must be clearly stated and outlined.

2) Laboratory records should be retained in a permanent file for a length of time set by government, other legal requirements, or the employing institution, whichever is longer.

3) Measurement results should be expressed so that their meaning is not distorted by the reporting process. Reports should make clear which results, if any, have been corrected for blank and recovery measurements and calibration conversions. Any other limitations should also be noted.

4) Data should be reported only to the number of significant figures consistent with their limits of uncertainty. The uncertainty should be stated to two significant figures and the reported value to the last place in the uncertainty statement.

5) When appropriate, the relationship between individual sample values, blanks, recoveries, and other supporting data should be shown. If possible, and within the scope of desired results, a number of measurements sufficient for statistical treatment should be made. When this is not the case, an explanation is necessary including complete details of the treatment of the data.

6) Data should be reported using S.I. units, where appropriate.

7) If a published methodology is used, it should be cited. Any modification, as well as any new methodology or new approach to the making of measurements or interpreting the results, must be described in detail, including test results and details of its validation. Instrumentation should be described.

8) If average values are reported, an expression of the precision, including the number of measurements, must be included. The minimum requirements for data to be used for statistical comparisions is the mean, standard deviation and number of replicates (Macdonald and Nelson, 1984).

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2. METHODS FOR THE ANALYSIS OF METALS IN SEDIMENTS, BIOTA AND INTERSTITIAL WATER

2.1 Introduction

2.1.1 The Metals and Environmental Media

This review includes those metals which are specifically mentioned in the Ocean Dumping Control Act (As, Be, Cd, Cr, Cu, Hg, Ni and V), and those which are useful for their interpretive or diagnostic value (Fe, Mn, Al and Ba). The two elements which have been responsible for the worst episodes of environmental metal poisoning, Hg and Cd, are limited to 0.75 and 0.6 µg/g (dry weight) respectively in dredge spoils and marine sediments by the Act. The toxicological significance of heavy metals in documented cases of metal poisoning of the aquatic environment have been described by Forstner and Wittman (1979). Iron and aluminum are often measured because they are major constituents of the aluminosilicate minerals which make up the bulk of marine sediments. The trace metals, which include all of the toxic metals of interest covered by this review, exist in characteristic ratios to the major elements in the rocks from which the sediments derive. Thus, anomalously high ratios indicate such unusual influences as natural ore bodies or anthropogenic sources. Barium is included in the list of metals because $BaSO_4$ is a major component of drilling muds. Because Ba is so abundant in drilling muds compared to marine sediments, it makes an excellent tracer of drilling mud accumulations on the seabed near offshore drilling sites (see section II-1.5).

Anthropogenic metals introduced in dissolved form are eventually precipitated through chemical and physical sorption processes. The anthropogenic metals thus exist in sediment in a more reactive form than metals that are contained in aluminosilicate minerals. Partial extractive techniques have been developed which dissolve the anthropogenic metals, along with naturally derived metals existing in the same form, but not the metals contained in minerals. Partial extraction methods are often useful in pollution studies and are included in the scope of this review. Natural and anthropogenic metal solids in sediments are sometimes mobilized by biochemical processes, such as reduction or methylation, that occur naturally in marine sediments. These processes (diagenesis) are responsible for the occurrence of high concentrations of dissolved metals in sediment interstitial (pore) waters. The presence of unusually high metal concentrations in interstitial waters may be responsible for the metals being more toxic to organisms living on or within the sediments.

Mercury and arsenic are known to exist in the marine environment as soluble organometallic compounds which are more toxic than their inorganic forms. Hence, methods have been developed to measure organomercury and organoarsenic species, and these methods are encompassed by this review. Lead is another metal which can exist in organometallic compounds in the marine environment, and methods exist for measuring them, but these have not been included in the review, since their only known source is tetraethyl lead added to gasoline. The evidence for natural methylation of lead in the marine environment is equivocal. Organoselenium compounds also exist in the marine environment, but selenium is not mentioned in the O.D.C.A., and so the review does not focus on it. However, methods for As, Se, Te, Bi and other metalloids are very similar and sometimes a method has been designed to measure several of these metals. Therefore, several methods for Se, Te, Bi, etc. occur incidentally in the review of methods for As. Similarly, methods for other metals not intentionally covered by this review, sometimes occur in methods for metals that are.

Methods are presented for three types of environmental media:

(1) whole sediments,
(2) benthic biota, and
(3) sediment interstitial waters.

Each material is sufficiently different from the others that separate methods have evolved. Sediments and biota must be extracted or dissolved before they can be measured by common instrumental techniques such as atomic absorption spectrometry. Seawater can be directly presented to the instrument (graphite furnace atomic absorption) when metal concentrations are high enough; otherwise solvent extraction (or some other technique) is used to preconcentrate the metals before instrumental quantification. The dissolution of solid samples can be accomplished with some combination of strong acids, which is usually different for sediments and biota. Complete dissolution of sediments requires HF since this is the only acid which will dissolve aluminosilicate minerals, whereas biota require a much more oxidizing combination of acids to destroy organic matter.

2.1.2 Instrumentation

The most widely used instrumental technique for determining trace metals in environmental materials is atomic absorption spectrometry (AAS). The reasons for this popularity are related to capital cost, ease of use, reliability and historical reasons. An atomic absorption spectrometer can be purchased for as little as $15-20K. The sensitivity of flame AAS is good relative to other instrumental techniques available, but it is sometimes not adequate for trace metals in marine samples, especially seawater. The sensitivity of AAS is increased by as much as 3 orders of magnitude with a graphite furnace attachment (which costs in the neighborhood of $10-20K). The sensitivity of graphite furnace-atomic absorption spectrometry (GF-AAS) is comparable to the most sensitive analytical techniques available, which are usually much more expensive and less available.

Inductively coupled plasma - atomic emission spectrometry (ICP-AES), a newer technique, is rapidly becoming established as a powerful alternative to AAS. Although it is more expensive (~$75K, minimum), it has the offsetting ability to make simultaneous determinations of many elements, whereas AAS must do the elements one at a time. It has the disadvantage of being a more sophisticated technique requiring more training to use. The sensitivity of ICP-AES is intermediate between flame and graphite furnace AAS, and the linear dynamic range of ICP much greater than AAS. A list of typical instrumental detection limits and sensitivities for the three instruments and a number of important elements is given in Table III-2.1. In addition to sensitivity and the cost advantages of multi-element analysis, many good analytical methods have recently been developed using ICP for marine samples. Methods based on ICP-AES generally offer a viable alternative to those based on AAS, and at the present time ICP is the second most useful instrumental technique for trace metal analysis of marine samples.

Atomic absorption spectrometry and atomic emission spectrometry are complementary techniques in the sense that they are subject to different kinds of interferences. With AAS, chemical and ionization interferences are common but spectral interferences are not. With ICP-AES, the opposite is true; thus if the determinations using both methods are in agreement, there is a high probability that the results are correct.

Several other instrumental techniques, though much less widely used than AAS or ICP, deserve mention. Foremost among these are the solid source methods, spark source mass spectrometry (SSMS), x-ray fluorescence spectrometry (XRF) and instrumental neutron activation analysis (INAA). The techniques have the advantage of avoiding a dissolution step when analyzing a solid sample; thus they make good reference methods. However, they are rarely used methods in marine analytical chemistry, either because they are relatively insensitive, too expensive, or

TABLE III-2.1

DETECTION LIMITS (μg/L) FOR FLAME AAS, GRAPHITE FURNACE AAS AND ICP-AES (from Floyd et al. (1980)(ICP) and Perkin Elmer (AAS))

	Flame AAS Detection limit[1]	Graphite Furnace AAS Detection Limit[1]	ICP-AES Detection Limit[2]
Al	20	-	-
As	50	1	10
Ba	8	0.2	0.5
Be	2	-	0.06
Cd	0.2	0.02	1
Cr	3	0.05	1
Cu	1	0.1	0.4
Fe	5	-	-
Hg	250	10	-
Pb	10	0.3	20
Ni	2	0.5	2
V	40	1	0.2
Zn	< 1	0.005	0.2

[1] Concentration for a S/N of 2

[2] Concentration for a S/N of 3

unavailable. These techniques are not included in this review. Differential pulse anodic stripping voltammetry (DPASV) has been used for determining Cu, Cd, Pb and Zn in seawater. It requires a small sample, is very sensitive, and can be used to determine labile species as well as total metal concentration (Batley, 1983). However, it is considered to be a research technique and is therefore excluded from this review. Gas chromatography is a very common instrumental technique for determining organic compounds in marine samples and is becoming widely used in inorganic analysis of organometallic compounds such as methylmercury.

2.1.3 Sources of Information

The following sources of information were accessed: pre-existing methods manuals, monographs, review articles, and the primary literature. The two main journals which contain methods for trace metal analysis of marine samples are Analytical Chemistry and Analytical Chimica Acta. A number of important method papers have appeared in oceanographic journals such as Marine Chemistry, Limnology and Oceanography and Marine Environmental Research, though these journals more often contain research reports where analytical methods have been applied. To facilitate finding all of the important methods papers, a computer search of the Chemical Abstracts data base from 1977-1982 inclusive was implemented (Can-Ole, C.I.S.T.I.). Analytical Abstracts was used to find many of the reports. Drs. J.A.J. Thompson and R.W. Macdonald (Institute of Ocean Sciences, Sidney, B.C.) provided useful bibliographies.

Because of the paucity of methods in some areas (e.g. trace metals in marine organisms) and in order to benefit from new developments in other fields, the scope of environmental media was often broadened to include geological and biological materials similar but not identical to interstitial waters, marine sediments and marine organisms. For example, methods developed for trace metals in foods and agricultural crops can often be applied to marine organisms. Since there are no methods that have been developed specifically for interstitial waters, it was necessary to review seawater methods to find suitable methods. In comparing analytical methods, it was assumed that desirable features could sometimes be abstracted from similar, compatible methods and incorporated in an improved method. This is often what analytical chemists do in adapting methods for use in their own laboratories. Standardization is generally lacking in marine analytical chemistry laboratories, and some development work is usually involved in adopting new methods. In comparing methods, simplistic or arbitrary conclusions were avoided, because ultimately a choice of method will involve a compromise between a number of competing factors which cannot be assessed in a review of available methods.

2.1.4 Reference Materials

Analytical quality control is a very important aspect of marine analytical chemistry and the reader should be aware of the standard reference materials (SRM's) that are available in this field. Russell (1984) has produced a booklet in which the available seawater, sediment and biological standard reference materials are described, trace metal concentrations given and the suppliers identified. The SRM's and their suppliers are given in Table III-2.2.

2.1.5 Organization of Methods

The review of methods is organized according to the type of sample and the elements that are determined. Many of the methods for Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni and V are multi-element methods, whereas those for arsenic and mercury are usually single element methods. Also, the common instrumental techniques for determining arsenic and mercury are unique in that they involve reduction to the hydride (arsenic) or atomic metal (mercury), which is determined in the vapour phase. Therefore, arsenic and mercury are reviewed separately for each sample type. At the end of each section in which existing methods for a given metal or groups of metals are described, the methods are summarized and compared, the relative merits of each and the factors affecting a choice of method are discussed.

TABLE III-2.2

STANDARD REFERENCE MATERIALS FOR METALS IN MARINE ANALYTICAL CHEMISTRY (from Russell, 1984).

	NAME	DESCRIPTION	SUPPLIER
SEDIMENTS	SRM 1645	River Sediment	NBS
	SRM 1646	Estuarine Sediment	NBS
	MESS-1	Marine Sediments	NRC
	BCSS-1	Marine Sediments	NRC
	NIES No. 2	Pond Sediment	NIES
	MAG-1	Marine Mud	USGS
BIOLOGICAL MATERIALS	SRM 1566	Oyster Tissue	NBS
	TORT-1	Lobster Hepatopancreas	NRC
	NIES No. 3	Chlorella	NIES
	NIES No. 6	Mussel Powder	NIES
	RM 50*	Albacore Tuna	NBS
SEAWATER	NASS-1	Seawater Reference Material for Trace Metals	NRC
	CASS-1	Material for Trace Metals	NRC

* Research material, values not certified.

ADDRESSES OF SUPPLIERS

National Research Council of Canada (NRC)

1. Atlantic Regional Laboratory
1411 Oxford Street
Halifax, N.S.
B3H 3Z1

2. Division of Chemistry
Montreal Road
Ottawa, Ont.
K1A 0R9

3. National Bureau of Standards (NBS)
Office of Standard Reference Materials
Washington, D.C. 20234
U.S.A.

4. National Institute of Environmental Studies (NIES)
Japan Environment Agency
P.O. Yatabe Tsukuba
Ibaraki 300-21
Japan

5. U.S. Geological Survey (USGS)
National Center
Stop 972, Reston, Va. 22092
U.S.A.

2.2 Interstitial Waters

2.2.1 Introduction

There are a number of problems associated with the determination of trace metals in marine sediment interstitial waters (Duinker, 1978). Although the concentrations of trace metals may be considerably higher than in seawater, allowing direct determination without preconcentration, concentrations are sometimes not higher, necessitating the use of methods that are sensitive enough to measure the low levels found in the water column. In these instances there is a problem adapting water column methods, which often require large volumes of seawater, to attain adequate sensitivity for the smaller sample volumes that are available from interstitial water samplers.

Besides sensitivity and limited sample, potential interferences are greater. It is well established that dissolved organic matter can complex a substantial fraction of trace metals in seawater (e.g., Van Den Berg, 1984; Huizenga and Kester, 1983) and this can be a serious interference for some analytical methods (e.g. Bruland et al., 1979). Decaying organic matter in sediments can cause interstitial water to be enriched in organic substances capable of complexing metals, resulting in a larger fraction of organically bound metals than occurring in the overlying seawater. Inorganic polynuclear complexes may also play a significant role in pore waters. The oxidation state of an element depends strongly on the Eh and pH of the solution and may be a critical factor in selecting an analytical method. The presence of H_2S in interstitial waters may result in the existence of insoluble or soluble metal polysulfide species not present in oxic seawater.

Finally, it has been found that many metals form organometallic compounds in sediments (e.g. Hg, As, Se, Te and Sn). Separate analytical methods such as GC-AAS have been developed to measure methylated species in seawater. In summary, the chemical complexity and compositional variability of interstitial water solutions creates problems of sampling, storage and analysis at least as problematic as those encountered for normal seawater. Much of this review, therefore, draws on the methods in use for trace metal analysis of seawater.

2.2.2 Sampling Interstitial Waters

In sampling and analysing interstitial waters for dissolved trace metals, one should exercise the same precautions to ensure that the results are not affected by contamination that are normally taken in the case of seawater. These include the use of clean room facilities, special purification procedures for labware and ultra-pure reagents. These problems and their solutions have been discussed by Batley and Gardner (1977) and Erickson (1978). Samples should be stored in rigourously cleaned polyethylene or Teflon bottles. Cleaning procedures are somewhat arbitrary. Patterson and Settle (1976) developed a cleaning procedure involving a series of acidic baths which typically provided a bottle blank of less than 1 ng for metals such as Pb. This procedure is time consuming (14 days) and may not be required for many applications. Cranston and Murray (1978) found that heating polyethylene bottles with 4 M HCl at 60C for four days was effective in producing a suitable bottle blank ($<$ 1ng) for Cr. The cleaning procedure needs to be adapted to the metal and the tolerable blank levels. High-purity acids and water must be used. Samples for Hg determination should be stored in glass (Pyrex quality) bottles unless an acidic oxidant is used as preservative, as Hg can diffuse through plastic. Bottles should be cleaned by a hot acid treatment to achieve a suitable blank (Agemian and Chau, 1976). To prevent adsorptive losses of metals to the container walls, samples are generally frozen or acidified with high purity acid to a pH of <1.8. This step can be omitted if samples are digested in the sample bottle before analysis.

There are some additional concerns that apply only to interstitial water, namely changes in metal concentrations caused by exposure to air or due to temperature change before and during extraction of interstitial water from the sediment sample. Kruikhov and Manheim (1982) have reviewed the principal methods of extracting pore water from sediment samples and discussed some of the potential problems involved in the various procedures. It is important to extract the sediment sample as soon as possible after collection, preferably in the field, taking care to minimize exposure to air and to keep the sample at the in situ temperature during storage and extraction.

There are two general approaches to sampling interstitial water in aquatic sediments. The first approach is to sample the sediments with a grab or core type sampler, then extract pore fluids from the sediment sample as soon as possible after collection. The second approach, which is an attempt to avoid some of the random or systematic errors inherent in the first approach, is to extract a sample of pore water from the bottom sediments with a specialized in situ pore water sampling device.

The two main techniques used in the first approach are:

1) pressure filtration, and
2) centrifugation.

Pressure filtration is the most widely practised technique. Variations have been tried utilizing mechanical, hydraulic and gas pressure. Manheim (1966) and Manheim and Sayles (1974) have described high pressure hydraulic type squeezers that have been employed with consolidated deep ocean sediments. For unconsolidated shallow water sediments, it is common to use squeezers powered by nitrogen gas pressures up to 1 MPa such as the one designed by Reeburgh (1967). Presley et al. (1967) reported a Reeburgh type squeezer made of Teflon for studies of trace metals in interstitial waters. For trace metal studies it is recommended that a rubber diaphram, sometimes used for distributing gas pressure, be avoided. Manheim (1972) has described a very simple technique for extracting small quantities of interstitial water from unconsolidated sediments utilizing a 50 cm^3 disposable plastic syringe. Vacuum filtration has also been used for separating water from near-shore surficial sediments, but it is much less efficient than positive pressure filtration.

Recently, centrifugation has been gaining favour as a technique for studying interstitial water chemistry (Edmunds and Bath, 1976). There are three variations in the basic technique. Interstitial water will rise to the top of the centrifuge tube for fine grained, unconsolidated sediments, but for coarse-grained sediments it is preferable to use a basal cup collection system. The third variation, recently studied by Batley and Giles (1980), is the most effective. This method utilizes a heavy, water-immiscible solvent 'Fluorinert FC-78' to displace interstitial water from the sediments during centrifugation. Batley and Giles (1980) compared the three centrifugation techniques and low pressure squeezing for efficiency and found that the solvent displacement technique gave the highest recoveries for sandy and clayey sediments. Low pressure squeezing was found to be less effective than solvent displacement centrifugation for extracting water from sandy sediments, while for clayey sediments the two methods were equally effective. The yields of interstitial water were improved by as much as one-third using the solvent displacement technique.

The problem of sampling interstitial water from sediments for trace metal analysis is essentially one of collecting and preserving the samples in conditions as close as possible to the natural ones. Centrifugation involves less sample manipulation than pressure squeezing, although precautions to avoid contamination are similar in both instances. Pressure effects are usually considered to be insignificant for low pressure squeezing devices. Temperature artifacts however, have been demonstrated (Fanning and Pilson, 1971), and exposure of anaerobic sediments, or pore waters derived therefrom, to air could alter the concentrations and physico-chemical forms of trace metals in the samples. This can be a serious problem where waters high in Mn^{2+} or Fe^{2+} are involved.

In situ sampling of interstitial water may subject the sediment-water system to less perturbation. Barnes (1973) and Sayles et al. (1976) have developed complicated in situ samplers mainly for sampling interstitial waters of deep sea sediments for the analysis of gases and major and minor constituents. The Barnes sampler is designed to be used as an outrigger attached to a core barrel and collects only one sample of interstitial water. The Sayles instrument functions like a large spring-driven syringe, withdrawing water from specific depths in the sediment column through ports located on the side of a 2 m length of aluminum tubing. Due to the materials used in their construction, neither sampler is suitable for trace metal work. Mayer (1976) and Hesslein (1976) have made very simple but effective devices from noncontaminating materials to recover pore waters in situ from lake or river bottom sediments. These samplers contain dialysis bags which are initially filled with distilled water. The sampler is thrust into the sediment column and left there long enough for equilibration to take place between inserted and ambient water - about one week. The samplers are easy to construct, and the idea could be adapted to marine trace metal studies. However, the dialysis membranes should be replaced with filter membranes to avoid possible exclusion of dissolved metal organic complexes, distilled water should perhaps be replaced with low blank seawater, and some thought be given to retrieval. The long time requirement is probably prohibitive for most routine applications.

2.2.3 Methods for Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

2.2.3.1 Direct-injection GF-AAS Methods

Direct injection of seawater into the graphite furnace of an atomic absorption spectrometer is an especially attractive method for interstitial water because it requires a minimal volume of sample.

There have been few applications of GF-AAS to direct determinations of metals in interstitial waters; most of the effort in this area has been focused on sea water. Brumsack and Gieskes (1983) successfully applied GF-AAS to the measurement of Fe, Al, Mn, Cr, V and Mo in pore waters from the Gulf of California. These authors give their detection limits and the instrumental parameters used for each element, but refer to other reports where the analytical methods are substantiated. Direct analysis of Fe and Mn in pore water by GF-AAS has been reported by Klinkhammer (1980b) who quoted estimated detection limits of 10 and 1 µg/L, respectively but does not give any other information about the analytical method or refer to its source.

Segar and Cantillo (1975) described methods for measuring Fe, Mn, Cu and Cd in seawater directly using the then newly introduced (Perkin-Elmer) HGA-2100 graphite furnace. Instrumental parameters are set and an appropriate volume of acidified seawater is injected. Standard additions is used to calibrate and matrix modifiers are not required. The effects of varying the instrumental parameters were thoroughly investigated and optimum conditions worked out for each element. It was found that the response for each element was very sensitive to salinity, particularly at the fresh water end of the scale. Segar and Cantillo also mentioned that the technique had been successfully applied to Zn, Cr and Ni. The approximate detection limits claimed for Fe, Mn, Cu, Zn, Ni, Cr and Cd are 0.4, 0.3, 0.5, .05, 3.0, 0.5 and 0.01 µg/L, respectively. The method used for obtaining the detection limits and how they are defined were not given, however.

Campbell and Ottaway (1977) developed methods for Cd and Zn based on the HGA-72 graphite furnace and 1 + 1 dilution of sample with deionized water with calibration by standard additions. The reported limits of detection for Cd and Zn were 0.04 and 1.7 µg/L, respectively.

Sturgeon et al. (1979) in collaboration with Guevremont et al. (1980) have elaborated on the methods of Segar and Cantillo, providing well-substantiated modified procedures for direct GF-AAS determination of Fe, Mn, Zn and Cd in seawater. Seawater is diluted 1+1 with deionized water containing NH_4NO_3 for the determination of Zn, and EDTA is used as a matrix modifier for determining Cd. The use of a modified graphite tube improves sensitivities for Fe and Zn. The sensitivity of determination for Fe in seawater is not different from that obtained from solutions of that element in deionized water, while the sensitivity for Mn and Zn is degraded by a factor of 2-3 by the seawater matrix. Calibration is by the method of standard additions. Sturgeon et al. (1979) have not determined detection limits due to the difficulty of measuring a blank for seawater; instead they have reported the analytical precision for replicate determinations on the same sample of seawater

(Table III-2.6). Guevremont (1980) extended his earlier work on Cd by trying various organic matrix modifiers.

Montgomery and Peterson (1980) developed methods for Fe, Mn and Cu in seawater based on direct GF-AAS. They found that the sensitivity is very dependent on the quality of the pyrolytic graphite coating on the graphite tubes which is degraded by the use of NH_4NO_3 as a matrix modifier. They recommend using a 1+1 dilution of seawater with deionized water and matrix matching of blanks and standards with a seawater low in Fe, Mn and Cu in place of NH_4NO_3. McArthur (1977) using uncoated tubes in an HGA-74 graphite furnace and a multi-element hollow cathode lamp has claimed a detection limit of 0.04 µg/L for Mn in seawater. Carnrick et al., (1981) investigated problems in the determination of Mn in seawater with a stabilized-temperature platform graphite furnace and Zeeman background corrector and found that less than 0.02 µg/L could be detected under optimum conditions. Halliday et al. (1980) reported a direct GF-AAS method for determining Pb in polluted seawater. The use of a tantalum coated tube and matrix modification with NH_4NO_3 largely removed interference caused by the major seawater constituents to achieve a detection limit of 1 µg/L, which compares well with that for Pb in pure solution. Pruszkowska et al. (1983) reported the determination of Cd in seawater with a platform graphite furnace and Zeeman background correction. The detection limit reported is 0.013 µg/L using 12 µL injections, which is about half what was previously thought to be the detection limit for Cd in pure water solutions with conventional background correction and very near to reported lower concentration limits for seawater.

Beatty and Cooksey (1978) described how to measure barium in seawater at the 10 - 20 µg/L level with good precision by direct injection into an HGA-500 furnace containing a pyrolytic graphite tube. The background absorption from the matrix is eliminated by the tungsten iodide source of the Perkin-Elmer Model 5000 AAS. Matsusaki et al. (1981) have investigated the mechanisms of interference caused by chloride and coexisting cations on the determination of Cr by GF-AAS. The addition of a masking agent, tetra-ammonium EDTA, removes the interference caused by chloride salts.

There is ample evidence that a number of trace metals can be determined in seawater and interstitial water by direct injection GF-AAS techniques. The direct methods are in general more rapid and require a smaller volume of sample than methods involving preconcentration. There are a number of possible disadvantages, however. The most important question regarding direct injection /GF-AAS methods is sensitivity. Comparing the detection limits for the methods that have been reviewed with the concentration ranges for open ocean seawater given in Table III-2.3 it is clear that in general, the methods are not suitable for open ocean samples. There are few published data for interstitial waters. However, the data given in the references cited suggest that concentrations vary from normal seawater concentrations to values that are several hundred times greater. Thus the detection limits for direct injection methods may be adequate for many interstitial water samples, but not enough is known about the occurrence and distribution of trace metals in interstitial waters to predict for which metals and in what sediments.

The direct injection methods sometimes demand a level of instrumental sophistication that is uncommon in routine laboratories. Better results are likely to be obtained with more modern and expensive equipment containing such features as Zeeman background correction, advanced temperature control systems, specially modified or coated graphite tubes or the L'vov platform. The direct methods are also generally dependent on the equipment used; problems or success obtained with a graphite furnace of one design cannot be automatically extended to a system with a different design. Simultaneous background correction is invariably used to cope with the seawater background. If the background correction is inadequate, a spurious positive or negative signal will result leading to an error when the method of standard additions is applied. Usually it is recommended to make standard additions to every sample; this makes the analysis take 3 times longer than if a standard calibration curve is used. Matrix modifiers such as NH_4NO_3 can be used to eliminate some of the background absorbance, but they can also contribute substantially to the blank and seriously degrade the performance of graphite tubes after relatively few injections. Most of the papers have given methods for one or two metals. Each metal presents unique problems and requires a unique set of graphite furnace conditions. These facts suggest that direct injection methods are more difficult to apply in practice than one might expect. They may also be prone to poor reproducibility and systematic error.

2.2.3.2 Preconcentration GF-AAS Methods

a) Multi-element Methods

Preconcentration is necessary to achieve greater sensitivity for trace metal determinations in seawater by AAS. The usual techniques are 1) solvent extraction of metal chelates, 2) coprecipitation by an organic chelator and 3) adsorptive

TABLE III-2.3

RANGE OF METAL CONCENTRATIONS (ng/L) FOR OPEN OCEAN SEAWATER

(from Burton and Statham, 1982)

Metal	Concentration Range
Fe	100 - 1000
Mn	30 - 3000
Al	500 - 5000
Cu	60 - 600
Zn	10 - 600
Ni	140 - 1800
V	700 - 1400
Cr	50 - 250
Cd	5 - 100
Pb	1 - 15
Hg	4 - 10
As	750 - 2000
Ba	4000 - 14000
Be	1 - 30

concentration by a chelating ion exchange resin. Whichever preconcentration method is employed, the graphite furnace is definitely superior to flame AAS for the final determination, since the absolute (mass) sensitivity is of prime importance in analysing small samples of interstitial water.

1) Coprecipitation

The coprecipitation method of Boyle and Edmond (1977), modified for 0.5 -1.0 mL aliquots of interstitial water, was applied by Klinkhammer (1980b) to measure Fe, Ni and Cu in sediments from the Eastern Equatorial Pacific. Recoveries of 90% and precisions of about 10% are claimed. In a later study (Klinkhammer et al., 1982) 1 or 5 ml aliquots of seawater were preconcentrated to 0.2 ml using the same techniques. The blanks reported are: 2 ng/L for Cd, 38 ng/L for Cu and 35 ng/L for Ni. The blank for Fe was so high that it obscurred any systematic trends in the samples. The method of Boyle and Edmond (1977) is based on an earlier method (Boyle and Edmond, 1975) in which Cu, Ni, Cd and Fe are coprecipitated with cobalt pyrolidinedithiocarbamate and measured by GF-AAS against blanks and standards prepared in the same matrix. The reported concentration ranges and standard deviations for samples from the open sea are 0.06 - 0.36 ± 0.006 µg/L for Cu, 0.17 - 0.61 ± 0.02 µg/L for Ni and 0.0 - 0.1 µg/L for Cd. Recoveries are essentially quantitative for Cu and Ni, whereas Cd recovery is about 10% low. It was noted that cobalt interferred with the determination of Cd, and this interference was removed by a second atomization (cleaning) cycle. Sensitivities were enhanced by minimizing the amount of cobalt carrier used for coprecipitation. The complete procedure requires about 5 hours, but many samples can be run in one day. Klinkhammer's "micro" modification of the method collected the pecipitate by centrifugation instead of filtration employed by Boyle and Edmond. Sawlan and Murray (1983) also applied these methods to the study of red clay and hemipelagic marine sediments. Their blanks for Cu and Ni were less than 0.18 and 0.24 µg/L, respectively.

2) Chelation Solvent Extraction

The chelation solvent extraction methods of Danielsson et al. (1978) and of Bruland et al. (1979) have proven to be capable of reliably measuring trace metals in seawater from the open ocean. In the method of Danielsson et al., Cd, Co, Cu, Fe, Ni, Pb and Zn are extracted from 500 mL aliquots into Freon TF by a mixture of ammonium pyrolidinedithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDTC). The metals are then back-extracted into concentrated nitric acid and diluted to 10 ml; the resulting solution of metals, which is 50 times more concentrated and free from major constituents, is well suited for analysis by the graphite furnace. The major advantages of Danielsson's extraction method over the older extraction methods (e.g., Brooks et al., 1967) are that metal concentrations in the final solution do not change with time and higher extraction efficiencies can be attained with two extractions instead of one. The recovery of spikes is greater than 90% for all of the metals. However, extraction of iron without prior acidification to dissolve colloidal species yields low results. The addition of EDTA to seawater in amounts greater than natural organic chelators did not affect extraction yields. Calibration is by the method of standard additions. Magnussen and Westerlund (1981) investigated the two step extraction process in greater detail using three different organic solvents. It was found that Cd, Cu, Fe, Ni, Pb and Zn could be extracted quantitatively using Freon TF, chloroform or methylisobutyl ketone (MIBK), whereas cobalt could be rapidly back-extracted only from Freon and chloroform. MIBK also has the disadvantage that small quantities of seawater are extracted along with the metal dithiocarbamates, leading to matrix interference problems in the final extract. The disadvantage of Freon is the lower solubility of metal dithiocarbamates in that solvent compared with chloroform and MIBK, which limits the maximum concentrations of metals that can be extracted quantitatively. Danielsson et al. (1982) tested and applied the Freon extraction method to estuarine waters which usually contain higher concentrations of metals. The solubility of metal dithiocarbamates in Freon was not found to be limiting. The method gave complete separation of analytes from the matrix irrespective of salinity, confirming its usefulness over the entire salinity range of an estuary. However, incomplete extraction, especially for iron, was obtained for low salinity samples which had been pre-acidified to 0.014 M with HNO_3 and stored in polyethylene bottles at room temperature for one week. Quantitative extraction was obtained by pretreating the acidified sample to release metals from unreactive complexes, either by heating for 24 hours at 60°C or by autoclaving for one hour after adding 0.5 mL of 30% H_2O_2.

Danielsson's Freon extraction method has been further validated in a study by Brugmann et al. (1983). During a cruise to the North Sea and to the N.E. Atlantic, 90 samples of seawater were obtained from various depths. Three different methods of analysing the samples were applied and the results compared. Cadmium, Cu and Pb were determined by an ASV method onboard ship. A modified APDC-MIBK extraction, after Brooks et al. (1967), was performed on the ship; the extracts were later analysed for Cd, Cu, Ni, Pb and Zn by GF-AAS in the clean-room laboratory at the University of Goteborg. The Freon extraction method of Danielsson et al. (1978, 1982) was also applied in the clean-room laboratory to preserved aliquots of the seawater samples. The Freon extraction method generally gave better precision for replicate determinations than the MIBK extraction, probably because of the difference in laboratory conditions in which the extractions were performed. The Freon extraction method also gave much lower blanks and thus lower detection limits. Blanks and detection limits for the three methods are given in Table III-2.4. All three methods proved to be capable of measuring the very low concentrations of metals in these samples, which lie at the lower limits of the ranges given in Table III-2.3. Generally small but statistically significant discrepancies occurred for the three methods. The reasons for these are not obvious, and the accuracy of the methods can not be estimated without analysing a sample of standard reference seawater or by applying an 'absolute' method of analysis.

The method of Bruland et al. (1979) is similar to that of Danielsson et al. (1978). Copper, Cd, Zn and Ni are extracted from a 250 ml aliquot of seawater into chloroform by a mixture of APDC and DDTC. Nitric acid is again used to back-extract the metals. The back-extract is evaporated to dryness and the metals are re-dissolved in about 1.25 mL 1M HNO_3, achieving a concentration factor of 200. This procedure often produces a solution in which Cd and Zn are too concentrated to be determined directly by GF-AAS, but Cu and Ni concentrations are optimal. Recoveries are quantitative and blanks are very low. Bruland et al (1979) field tested the method by analysing samples taken from a vertical profile in the California Current off the coast of Central California. Samples were also analysed by a Chelex 100 ion exchange concentration method modified from that of Riley and Taylor (1968). The blanks and detection limits for both methods are given in Table III-2.4. The chloroform extraction method and the Freon extraction method have comparable blanks.

Smith and Windom (1980) developed solvent extraction procedures for Cd, Cu, Zn and Ni based on the dithizone/chloroform and dimethylglyoxime/chloroform systems which when combined with GF-AAS gave blanks and detection limits comparable to those of Danielsson and Bruland. The methods were applied to samples of fresh water and seawater, and good precision and recoveries were obtained.

TABLE III-2.4

BLANKS AND DETECTION LIMITS OF PRECONCENTRATION/ GF-AAS METHODS FOR SEAWATER (values are given in ng/L)

	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Cd	< 0.2	3 (1)	2	<0.2(.2)	<0.6(.6)	< 0.2
Cu	< 1	15 (5)	10	23(10)	4(6)	< 0.4
Ni	< 10	20 (8)	-	< 8(10)	40(15)	< 6
Pb	< 1	8 (2)	1	-	-	< 1.6
Zn	< 5	50 (15)	-	2(2)	5(15)	4

Method 1 - Freon extraction blank, Brugmann et al. (1983)

Method 2 - MIBK extraction blank (standard deviation), Brugmann et al. (1983)

Method 3 - ASV method "estimated detection limit", (undefined), Brugmann et al. (1983)

Method 4 - Chloroform extraction blank (detection limit*), Bruland et al. (1979)

Method 5 - Chelex preconcentration blank (detection limit*), Bruland et al. (1979)

Method 6 - Immobilized 8-hydroxyquinoline on silica gel extraction blank (Sturgeon et al. 1981)

*Defined as 2.5 x S.D. of the blank

3) Chelating Ion Exchange

In terms of blanks and detection limits, the Chelex method used by Bruland et al. (1978) compares well with the chloroform extraction method. However, the Chelex method gave substantially lower values for Cu and Ni than those obtained from the chloroform extraction method. This discrepancy was explained by the inability of Chelex to retain metal ions associated with colloidal and fine particulate matter, in agreement with the earlier assessment by Abdullah et al. (1976) who found that quantitative removal of Cu by Chelex 100 required prior acidification and heating. Another disadvantage of the Chelex method is that large amounts of Ca and Mg are retained on the resin and are co-eluted along with the trace metals in the acid. This creates matrix interference problems and requires that blanks and standards are made up in a matching matrix of purified alkali metal and alkaline earth salts. Kingston et al. (1978) circumvented this problem by eluting alkali and alkaline earth metals with ammonium acetate before eluting the trace metals with nitric acid. The Chelex method of Riley and Taylor was used by Hartmann and Muller (1982) to measure Mn, Cu, Zn and Ni in 200 - 300 ml aliquots of interstitial waters from Central Pacific Ocean sediments. Rasmussen (1981) compared the Chelex and Freon extraction methods using samples of seawater taken from the Baltic Sea. He concluded that preconcentration by the extraction procedure proved to be more reliable and robust than the Chelex 100 procedure.

Immobilized chelates are finding increasing application for preconcentrating trace metals from seawater. A method for determining Fe, Mn, Cu, Zn, Ni, Co, Pb and Cd which utilizes 8-hydroxyquinoline (oxine) chemically bonded to silica gel has been developed by Sturgeon et al. (1981). Aliquots of seawater (500 -900 mL) are drawn through a specially prepared column at 15 mL/minute; the column is then rinsed with de-ionized water and the metals are eluted with 10 ml of 2N HCl/0.1N HNO_3. Quantitative recovery of the metals (except for Co which was 74%) was verified by analysing samples of seawater by several different methods. The precision averaged 6% for all metals and concentrations. The reported blanks are comparable to those given for the other methods (Table III-2.4). The method gives essentially matrix free concentrates suitable for instrumental analysis. Other advantages are that seawater can be processed in much less time than using Chelex 100, and very high concentration factors (500 fold) are feasible.

b) Single Element Methods for Mn, Cr, V, Ba and Be

Manganese is not usually extracted from seawater using dithiocarbamate chelating reagents because their mutual affinity for Mn is relatively small. A complete method for determining Mn in seawater has been given by Klinkhammer (1980a) which utilizes 8-hydroxyquinoline (oxine) to extract Mn into chloroform. The Mn oxinate is then back extracted into 3M HNO_3 and determined by GF-AAS. The accuracy and precision claimed for this method is 9%, and the average total blank is about 3 ng/L. This method has the disadvantage of low efficiency (about 70%) for the overall extraction, which must therefore be monitored by counting the gamma activity of spiked Mn-54. Klinkhammer (1980b) has used this method for studying interstitial waters. A spectrophotometric method for Mn, which involves formation of a reddish-brown complex with formaldoxime, is given by Koroleff (1983) (concentrations as low as a few µg/L can be measured). Iron can also be determined by colorimetry by the ferrozine method of Stookey (1970) or by the TPTZ method given by Koroleff (1983). The lower limit of determination for the latter method is about 1 µg/L.

The determination of chromium in seawater is complicated by the fact that it exists in two forms, Cr (III) and Cr (VI), mostly the latter, and methods have thus been developed to measure both forms. Two different methods are available for determining Cr species in seawater based on flameless AAS with preconcentration. The method of Cranston and Murray (1978) which precipitates either Cr (III) or both species of chromium with $Fe(OH)_3$ has proven to be useful. The method depends on independent measurements of two equivalent samples. Hydrous Fe (III) oxide precipitates only Cr (III), while Fe (II) hydroxide reduces hexavalent chromium at pH 8 while itself being oxidized to ferric hydroxide, thus precipitating all chromium from the sample. Chromium (VI) is determined by difference. Recoveries are adequate (80 -90%) and selectivity for Cr (III) is good. The method is sensitive enough using 140 mL samples to determine Cr in the open ocean. The separations can be performed shipboard, which minimizes changes in speciation during storage, and about 20 samples can be processed in one day.

The other method, that of de Jong and Brinkman, (1978), selectively extracts either trivalent or hexavalent chromium from seawater using a liquid cation exchange medium, Aliquat - 336. Hexavalent chromium is extracted at pH 2, whereas trivalent chromium is extracted from a neutral solution of sample containing 1M KSCN. This method has a low blank and detection limit for 500 mL samples, good selectivity for added Cr (III) and (VI), and has the advantage of determining both forms directly. Total chromium can also be measured by first oxidizing Cr (III) with persulfate and extracting all Cr as Cr (VI) at pH 2. Comparison of Cr (III) obtained by direct extraction and by the oxidation method revealed that in some samples of coastal water from Holland most of the Cr (III) is present as highly stable organic complexes. A disadvantage of de Jong and Brinkman's method is that it is not convenient to perform the extractions and the final determination by GF - AAS in the field. Storage of the samples is not satisfactory either, since changes in the distribution of species is likely. Campbell and Yeats (1981) applied the de Jong and Brinkman method to measure total chromium for vertical profiles in the Northwest Atlantic Ocean.

Two additional methods have been developed which are suitable for determining total chromium in seawater. Willie et al. (1983) recently reported a method which utilizes silica-immobilized diphenylcarbazone to preconcentrate chromium from seawater for determination by GF - AAS. All chromium is first reduced to the trivalent form by addition of SO_2-water to the sample. A 200 mL aliquot is then adjusted to pH 9.0 and passed through the diphenylcarbazone column at a rate of 10 mL/minute, interstitial seawater is washed out with de-ionized distilled water and chromium is eluted from the column with 10 mL of 0.2M HNO_3. Recovery is quantitative, though sensitive to pH. The blank and lower limit of determination using 200 mL samples are adequate for determining total chromium in samples from the open sea. Additional gains are possible using larger samples of seawater. The column has a relatively small exchange capacity, which may place some limits on the applicability of the method. Also, colloidally bound Cr (III) may be inert to the column. Chromium can be extracted from seawater using the APDC - MIBK system (Bergmann and Hardt, 1979). The sample is mixed with APDC and potassium hydrogen pthalate and heated for 20 minutes at 80 C before being extracted with MIBK; the organic layer is directly analyzed by graphite furnace. Both Cr (III) and Cr (VI) are equally well extracted and they exhibit equal AAS sensitivity.

Several single element methods for determining vanadium in seaweater by AAS exist. Morris (1975) measured vanadium on a section in the Northeast Atlantic Ocean by a method based on Chelex 100 and flame AAS. The method of Monien and Stangel (1982) involves preconcentration by solvent extraction and determination of V in the extract by GF - AAS using a pyrolytic graphite tube pre-coated with lanthanum carbide. A coefficient of variation of 7% for 1 μg/L concentrations is claimed.

Shimizu and Sakai (1981) have advanced a method of coprecipitating vanadium from seawater with $Fe(OH)_3$ - hydrated TiO_2 at pH 6 followed by digestion and determination by GF - AAS. For seawater containing 1.7 µg/L of vanadium, the coefficient of variation is 4.7%.

Rollemburg and Curtius (1982) reported a method for Ba whereby interferring ions in seawater are removed by chromatography on Dowex 50W-X8 resin. The detection limit is 9 µg/L. As little as 1 ng/L of beryllium in seawater can be detected by the method of Burba et al. (1983). Beryllium is preconcentrated by adsorption onto Cellulose - Hyphan or Cellulose - pyrogallol, eluted with 2M HCl and determined by GF - AAS. Beryllium can also be extracted by a solution of diphenyl-thiocarbazone, 8-quinolol and acetyl acetone in ethyl propionate at pH 5 - 7 (Sachdev and West, 1970). Recently, Measures and Edmond (1983) reported measuring beryllium in seawater by electron capture - gas chromatography of the extracted trifluoracetylacetonate complex.

c) Conclusions

In comparing multi-element preconcentration methods, the solvent extraction methods of Danielsson et al (1978) and of Bruland et al (1979) seem to offer the most advantages. They are based on reliably established and widely accepted techniques of solvent extraction into an organic solvent using a mixture of chelating agents, followed by back-extraction with nitric acid. Danielsson's and Bruland's procedures are very efficient. Preconcentration factors of several hundred can be achieved, bringing the analytes into the optimum range for GF-AAS. With an automatic sampling system, a coefficient of variation of 1-2% can be achieved for replicate injections of the same sample. The concentration of sea salts in the final extract is too low to interfere, and the methods can achieve extremely low blanks. The detection limits (determined by the variability of the blank) will be low enough for interstitial water samples provided that the solvent extraction is scrupulously performed in a clean-room laboratory. Recoveries are essentially quantitative; natural organic chelators generally do not interfere. However, in view of the recent findings of Danielsson et al. (1982), it is recommended that the wet oxidation procedure be applied to samples of interstitial water to check whether unreactive metal-organic complexes of metals exist.

The silica-bound oxine extraction method of Sturgeon et al (1981) is a very promising new technique. In terms of analytical performance (extracting efficiency, blanks, and detection limits), convenience and applicability it may surpass all the rest, but it is newer and therefore less proven. The diphenylcarbazone column method of Willie et al (1983) may have similar potential. The Chelex 100 technique has been in use for a long time, but it appears to give less efficient separation from sea salts, lower extraction efficiencies (recoveries), and therefore it is probably not capable of producing as high preconcentration factors. Also, Chelex extraction is more subject to interference from natural organic chelators than are the solvent extraction methods of Danielsson and Bruland. The coprecipitation method of Boyle and Edmond (1977) is less important than the solvent extraction methods, because it is only applicable to a few metals, it has higher blanks, and the cobalt used as a carrier can interfere with the AAS determination. However, it has the advantage that it has been sucessfully applied to interstitial water samples.

Applying seawater methods to interstitial waters involves scaling them to accommodate smaller sample volumes. Klinkhammer modified the coprecipitation method to analyze 0.5 - 5 mL samples of interstitial water; he used centrifugation instead of filtration to separate the precipitate and analyzed for 3 metals in a final volume of 0.2 mL. This is within the capability of GF-AAS, since 5 or 10 µL aliquots may be injected. There is no reason why the solvent extraction and chelating ion exchange techniques can not be scaled to the smaller volumes available for interstitial waters. In most cases minor modifications, such as the use of smaller separatory funnels, are all that is required. The volume of sample that is available for analysis depends on the technique used to extract a pore water sample from the sediments, which in turn depends on the nature of the problem being investigated. It may be as little as 0.5 mL or as much as 0.5 L; the pressure squeezers and centrifugation methods in common use would normally yield a volume in the range of 10-100 mL. The smallest practical final volume for multi-element GF-AAS analysis is probably 1.0 mL, which would permit preconcentration factors in the range 10-100 and would enable a number of metals to be determined within their oceanic concentration ranges.

The two classes of methods which involve preconcentration and direct injection of seawater into the graphite furnace cover about the same group of metals. In terms of sensitivity and detection limits, the direct injection methods are certainly inferior. The preconcentration methods effectively eliminate interference due to the major inorganic constituents of sea water but may be subject to interference from unreactive metal species or dissolved organic matter. These interferences are not prevalent for seawater, but interstitial waters may be more problematic. Therefore, a wet oxidation is recommended to check for possible incomplete extraction. Direct injection methods rely on specialized and sometimes sophisticated techniques to deal with interferences from the major constituents; it is not clear whether these interferences have been totally eliminated. For this reason the accuracy of the direct injection methods is more uncertain. When a preconcentration method simultaneously extracts 6 or 8 metals, there is not much time saved in applying direct injection methods for those metals, since the analysis by graphite furnace of sample concentrates is much more rapid. Both methods require careful technique and clean room conditions to obtain the best results, but the preconcentration methods are probably more prone to sample contamination due to more extensive sample manipulation.

2.2.3.3 ICP-AES Methods

Inductively coupled plasma atomic emission spectrometry (ICP - AES) has not yet been extensively applied to the analysis of seawater. The sensitivity of ICP -AES, though about an order of magnitude greater than flame AAS, is still not sufficient for determination of most trace metals in seawater without preconcentration. In addition, the high dissolved salt content of the seawater matrix is not compatible with the sample introduction systems used with ICP. Because ICP is a relatively new technique which is rapidly gaining acceptance, it is useful to consider several recently reported methods for the analysis of seawater for trace metals.

The method of Berman et al (1980) employs the Chelex 100 technique (Sturgeon et al., 1980b) to preconcentrate Fe, Mn, Cu, Zn, Ni, Cr, Co, Cd and Pb from 250 mL sized samples of seawater. Iron, Mn, Cu, Zn and Ni were determined with good precision in a sample of coastal seawater, while Cr, Co, Cd and Pb concentrations were below the levels at which reliable analysis can be performed. The Chelex method was limited to preconcentration factors of about 25 times; when larger volumes were processed precision was reduced.

Sugimae (1980), McLeod et al. (1981) and Miyazaki et al. (1982) used solvent extraction to preconcentrate the metals that were determined. Sugimae extracted 1 L samples of seawater with sodium diethyldithiocarbamate and chloroform at pH 6.3. The chloroform was evaporated, the chelates decomposed, and the metals redissolved in 25 mL of nitric acid. The final metal concentrations were thus 40 times higher than the original ones. Quantitative recoveries were shown for Fe, Mn, Cu, Zn, Ni, Cd and Pb, while that was of V was 83%. McLeod et al. extracted 500 mL aliquots of seawater with APDC + DDDC into chloroform at a pH of 4.1. The chloroform was back-extracted into nitric acid and evaporated in a Teflon beaker. The final solution was 500 times more concentrated in Cd, Cu, Fe, Mo, Ni, V and Zn than the original seawater, resulting in greatly improved detection limits. Miyazaki et al. extracted Fe, Mn, Cu, Zn, Ni, Cd, Pb, Mo and V from 500 mL of seawater with APDC and di-isobutyl ketone (DIBK) at pH 2.4, achieving a concentration factor of 100. The organic extract was directly nebulized into the plasma. DIBK was chosen for the purpose after evaluating more than thirty aliphatic and aromatic liquids for ease of introduction into the ICP and aqueous solubility. Some of the solvents, particularly those with high vapor pressure, caused fluctuation and mismatching of the plasma. The limits of determination, defined as the concentration necessary to produce a signal equivalent to ten times the standard deviation of the background signal are given for the three methods in Table III-2.5.

TABLE III-2.5

LIMITS OF DETERMINATION (ng/L)* OF ICP METHODS FOR SEAWATER

	Method 1	Method 2	Method 3
Fe	250	230	30
Mn	60	-	-
Cu	500	150	10
Zn	130	250	10
Ni	500	340	80
Pb	2500	180	-
Cd	250	70	10
Mo	-	600	60
V	380	110	30

Method 1 - Sugimae (1980)
Method 2 - Miyazaki et al (1982)
Method 3 - McLeod et al (1981)

* Concentration of metal in orignal seawater which gives a signal equivalent to 10 times the standard deviation of the background signal. Concentration factors for preconcentration steps are as indicated in the text.

It is obvious that the limits of determination depend strongly on the preconcentration factor. These figures, being based on the instrumental detection limits, do not necessarily reflect the precision of the blank determination and may thus be somewhat misleading. McLeod et al. also determined their blanks using GF - AAS, which are comparable to those given in Table III-2.4 for the solvent extraction/GF -AAS methods. Judging from this and the fact that the limits of determination for the McLeod et al. method are similar to those that can be inferred from the data in Table III-2.4 for the solvent extraction/GF-AAS methods, it is clear that the ICP-AES methods can approach the sensitivity of the GF-AAS methods when extremely high preconcentration factors are effected. By comparing the limits of determination for the method of McLeod et al. with the concentration ranges given in Table III-2.3, it can be concluded that with the exception of cadmium, the ICP method of McLeod et al. is sensitive enough to measure trace metal concentrations in the ocean. To obtain that kind of sensitivity however, the method requires at least 500 ml of seawater, which is impractical for interstitial water studies. For relatively abundant elements in sediment it may be possible to use ICP directly. Brumsack and Gieskes (1983) determined barium in diluted interstitial water by ICP by adding a known concentration of iron to the samples as an internal standard. No further information on this method was given.

Solvent extraction follwed by ICP determination is a promising technique for seawater and interstitial water analysis, but it is still a relatively new approach. The solvent extraction method used by McLeod et al is essentially that of Bruland et al (1979) which has proven to be efficient and reliable. In terms of sensitivity, ICP is intermediate between flame and graphite furnace AAS. In order to achieve comparable detection limits, a larger preconcentration factor must be employed, which may be a significant disadvantage for interstitial waters since it may be difficult to obtain large enough sample volumes. However, ICP determines a large number of elements (7-9) simultaneously on a single volume of extract (a few mL is sufficient), which results in a considerable time savings over GF-AAS.

2.2.3.4 A Comparison of Methods

The Marine Analytical Chemistry Standards group of the NRC (Ottawa and Halifax) made a comparative study of five different analytical methods for determining trace metals in seawater (Sturgeon et al., 1980b). Method 1 was the direct injection / GF - AAS method of Sturgeon et al. (1979). Methods 2 and 3 are based on GF - AAS with preconcentration by solvent extraction and chelating ion exchange, respectively. Method 2 (Sturgeon et al. 1980a) employs a mixture of APDC and oxine as chelating agents. The metals are concentrated 33 times by two successive extractions into MIBK and 1.5 M HNO_3. Method 3 (Sturgeon et al., 1980b) employs Chelex 100 resin, 1.0 M NH_4COOCH_3 to remove alkali and alkaline earth metals, and 2.5 M HNO_3 to strip trace metals from the resin. The resulting solution is virtually matrix free and about 20 fold preconcentrated. Method 3 is similar to that reported by Kingston et al. (1978). Method 4 (Berman et al., 1980) uses the same method of preconcentration as Method 3 with quantification by ICP - AES. The standard addition approach to calibration was used for Methods 1 - 4 to compensate for physiocochemical interferences. Finally, Method 5 (Mykytiuk et al., 1980) involves equilibration of isotopic spikes added to seawater before preconcentrating by the Chelex 100 method, and final quantification by spark source mass spectrometry. The results of applying the methods to two samples of coastal seawater are given in Table III-2.6.

Overall there is good agreement among the five analytical methods. The analytical precisions are generally comparable. This does not mean that the limits of detection are comparable, however. Although Fe, Mn, Cd, and Zn can be determined by Method 1 (GF - AAS without preconcentration) with precision comparable to Methods 2 and 3 (GF - AAS with preconcentration), Cu, Ni, Pb, Cr, and Co cannot be measured by Method 1 due to insufficient sensitivity. Similarly, Method 4 (ICP - AES with preconcentration) precisions are comparable to Methods 2 and 3, but Pb, Cd, Cr and Co cannot be measured because their levels in the samples are below those at which reliable analyses can be performed. Unfortunately, the authors of these studies did not determine detection limits for the five methods, probably because it is usually rather difficult to determine a "true" blank for trace metals in seawater. The two seawater samples used in these studies were of coastal seawater and are at or above the upper limits of the ranges for typical open ocean samples. One can get a rough idea of the lowest concentrations at which these metals can be reliably determined by these methods using the approach suggested by Sturgeon et al. (1979a). The 'limit of determination' as defined by Sturgeon et al. is the concentration of analyte in seawater giving a signal equivalent to twice the standard deviation of the determination, which differs from the definition of this term used earlier. However, it can be concluded from comparing the limits of determination derived from Table III-2.6 with the concentration ranges in Table III-2.3, that reliable determinations could probably be made at the lower oceanic concentrations for Fe,

TABLE III-2.6

RESULTS (μg/L) OF ANALYSIS OF TWO SAMPLES OF COASTAL SEAWATER BY FIVE DIFFERENT METHODS

(N.B. Methods 1, 2, 4 and 5 were applied to the same sample of seawater; Method 3 was applied to a different sample.)

Methods	Fe	Mn	Cu	Zn	Ni	Pb	Cd	Cr	Co	U
1	1.6 ± 0.1	1.2 ± 0.1	-	1.7 ± 0.2	-	-	0.20 ± 0.04	-	-	-
2	1.5 ± 0.1	1.3 ± 0.1	0.6 ± 0.2	1.9 ± 0.2	0.33 ± 0.08	0.22 ± 0.04	0.24 ± 0.04	-	-	-
3	3.4 ± 0.4	2.2 ± 0.3	0.51 ± 0.03	2.0 ± 0.1	0.45 ± 0.05	0.10 ± 0.1	0.053 ± 0.007	0.25 ± 0.02	0.018 ± 0.008	-
4	1.5 ± 0.6	1.5 ± 0.1	0.7 ± 0.2	1.5 ± 0.4	0.4 ± 1	-	- - -	-		
5	1.4 ± 0.1	1.8 ± 0.2	0.7 ± 0.1	1.6 ± 0.1	0.37 ± 0.02	0.35 ± 0.03	0.28 ± 0.02	0.34 ± 0.06	0.020 ± 0.003	3.0 ± 0.3

Method 1 - Direct GF - AAS, Sturgeon et al. (1979), Guevremont (1980)
Method 2 - Solvent extraction/GF-AAS, Sturgeon et al. (1980a)
Method 3 - Chelating ion exchange/GF-AAS, Sturgeon et al. (1980b)
Method 4 - Chelating ion exchange/ICP-AES, Berman et al. (1980)
Method 5 - Chelating ion exchange/ID-SSMS, Mykytiuk et al. (1980)

Cu, Ni, Cr, and Cd using some of these methods. The lower oceanic concentrations of Pb, Zn and Mn however, may be beyond the capability of all of these methods.

The fact that the various methods generally agreed attests to the accuracy of the individual methods, and indicates that the standard additions approach effectively compensated for the interferences arising during sample preconcentration and instrumental analysis observed in the development of the methods. Sturgeon et al. note that no problems were apparent in the solvent extraction or ion exchange preconcentration methods arising from non-labile metal species. This may have been a fortuitous result of the complete release of the natural metals from colloidal organic and inorganic forms during prolonged storage of the seawater at pH 1.6.

In comparing the five analytical methods, Sturgeon et al. state that direct analysis of seawater by GF - AAS is fast and accurate for Fe, Mn, Zn and Cd when the concentrations are high enough. When the concentrations are lower, solvent extraction/GF - AAS or ion exchange/GF - AAS can be used, the former being more useful when sample volumes are lower (about 100 mL). Ion exchange can be used if greater preconcentration is necessary (e.g. when ICP - AES is used in place of GF - AAS), but is more laborious than solvent extraction. ICP - AES has the advantage of being a multi-element technique, but has inferior detection limits to GF - AAS. Method 4 could probably be extended to include Cd, Pb and Co if greater volumes of seawater (hence greater pre-concentration) were processed by ion exchange. Interstitial water volumes are usually low, however. Isotope dilution SSMS offers multi-element capability and high sensitivity, but requires a large capital outlay and can only process relatively few samples per day. Because it is an 'absolute' method however, it is an excellent choice for reference determination. The results of any two different methods in combination would serve as a check, since different chemical and instrumental techniques usually lead to different errors (interferences).

2.2.4 Methods for Mercury

Burton and Statham (1982) have reviewed some of the more recent determinations of mercury in the ocean. The view that typical mercury concentrations in open ocean waters are substantially below 10 ng/L is well substantiated by studies reported since 1975. In coastal regions a considerable proportion of mercury is present in a form which is analytically unavailable without oxidative pretreatment of the sample.

The results of the last international ICES inter-laboratory comparison for the analysis of mercury in seawater have been reported by Olafsson (1982). Thirty-two laboratories from 16 countries participated in the exercise. Three samples were analysed by each laboratory - one natural sample and two that were spiked with $HgCl_2$ at 15 and 143 ng/L. Almost all of the laboratories used the $SnCl_2$ reduction method with detection by cold vapour AAS. Other methods included ASV and atomic fluorescence spectrometry. The majority of laboratories were able to determine with good accuracy and precision the recovery of the spikes, but systematic errors in the low level determinations were apparent which resulted from the actual concentration of mercury being at or very near the detection limits of the methods employed. The determined concentrations of mercury in the natural sample range from 0.76 to over 10 ng/L; the reported detection limits of the methods used range from 0.2 to 50 ng/L.

Kaiser and Tolg (1980) briefly reviewed the instrumental techniques of analysis for mercury in environmental samples. Burrows (1975) gave a detailed review of the status of total Hg determination up to 1975. By far the most popular and widely used instrumental technique is AAS. Flame and graphite furnace atomizers can be employed, but better sensitivity is achieved by the cold vapour atomic absorption (CV-AAS) technique for which detection limits in the range 0.5 - 5 ng are possible. There are many variations, but the basic technique involves converting all forms of mercury in the sample to the mercuric ion, reducing Hg^{2+} to Hg^0, partitioning the elemental mercury into the gas phase and measuring the atomic absorption at 253.7 nm in a quartz cell. To attain the highest sensitivity needed for seawater analysis, a gold or cold trap is placed in the gas stream and the atomizer cell to preconcentrate the mercury vapor. Fitzgerald et al. (1974) and Olafsson (1974) introduced this technique for determining total and reactive mercury in seawater.

Olafsson (1983) gives the latest version of his method in Methods of Seawater Analysis. Reactive mercury is determined by the addition of $SnCl_2$ to a 400 ml sample of acidified seawater. The liberated mercury is carried by an argon gas stream into a mercury collector consisting of a quartz tube filled with about 20 g of gold foil and wrapped with a resistance wire. A small current is applied to the wire while purging the sample with argon to prevent moisture from condensing on the gold. After 45 minutes the argon flow is increased and the resistance wire is heated to a dull red glow, releasing the adsorbed Hg. The mercury vapour is swept into a 20-30 cm path length optical cell of an AAS or a non-dispersive mercury monitor. The maximum absorbance, which appears in about a minute, is measured at 253.7 nm. To measure total mercury, the organomercurials are oxidized by adding $KMn0_4$ and $K_2S_20_8$ to the acidified sample, heating for 2 hours at 80°C, and then adding hydroxylamine prior to the $SnCl_2$. Standards and blanks are measured using acidified deionized water instead of the sample. The precision of the method is about 16% at 2.5 ng/L and 4% at 18 ng/L. Typically, blank levels are < 0.5 ng for all reagents used.

Bloom and Crecelius (1983) have used a variation of the two stage gold trap method of Fitzgerald and Gill (1979) to determine the levels of 'easily reducible' and total mercury in Pacific Northwest coastal waters, which are 0.1 - 0.5 ng/L and 0.2 - 1.0 ng/L, respectively. In the two stage gold trap method, mercury is collected on the first trap, then transferred to the second trap, and finally swept into the CV-AAS analyzer for measurement. The advantages are that virtually all interferences due to organics or chlorine are eliminated, and the precision and detection limits are improved. Radiotracer studies have shown that the mercury is quantitatively transferred to the second trap and the analyzer. Bloom and Crecelius also describe advances in the use of extremely clean reagents and special sample handling techniques that are responsible for making the blank lower and more reproducible. With these improvements, reliable detection of Hg in seawater at concentrations of 0.1 ng/L can be achieved. Accuracy and precision were demonstrated by the use of HNO_3 digested biological and geological standards, as well as a collaborative measurement of natural seawater. Three different methods of oxidizing a seawater sample for the determination of total mercury were also compared. The bromine monochloride (cold oxidation) technique of Szakacss et al. (1980) was found to be superior to the $KMnO_4$ + $K_2S_2O_8$ (hot oxidation) technique and the acid ageing technique. Although not tested by Bloom and Crecelius, the ultraviolet photooxidation technique has also been applied to determining 'total' mercury in seawater (Fitzgerald, 1975). The CV-AAS technique can readily be used on board an oceanographic research vessel.

Mechanized methods for Hg analysis (based on peristaltic pump and manifold systems) do not normally have the sensitivity required for determining Hg in seawater, but one such method (Goulden and Anthony, 1980) holds some promise for interstitial waters. The method has a detection limit of 1 ng/L which is at least an order of magnitude improvement over most mechanized methods. To obtain such a substantial improvement in sensitivity, Goulden and Anthony investigated the factors affecting the partitioning of elemental mercury between the gas and liquid phase. It was found that partitioning at 90 C, drying with sulfuric acid, and measurement at room temperature gave the best results. In addition, possible interference from volatile organic compounds was avoided by the use of a combustion furnace. While the detection limit is not low enough for oceanic levels, it may be adequate for interstitial waters and some coastal waters. A particular advantage of the method is that it can determine 3 species of Hg: inorganic mercury, aryl mercury compounds such as phenyl (II) mercury chloride, and alkyl mercury compounds such as methyl mercury (II) chloride. Speciation of mercury is made possible by taking advantage of the fact that inorganic, aryl and alkyl mercury are reduced to elemental mercury under different conditions. Inorganic mercury is reduced to mercury with hydroxylamine EDTA in alkaline solution. Inorganic mercury and aryl compounds are reduced to mercury with EDTA and $SnCl_2$. Cadmium chloride and tin (II) chloride reduce all forms of mercury including methylmercury, to elemental mercury. The main advantages of automated methods are high sample throughput (20 samples/hour) and greater precision (in this case, 1.2% at 200 ng/L and 5% at 10 ng/L).

Only one example of a mercury determination in interstitial water has been found. Bothner et al. (1980) measured total mercury concentrations in interstitial waters separated from contaminated estuarine sediments from Bellingham Bay, Washington by a CV-AAS method (Bothner and Robertson, 1975) finding enrichments of 126 times.

2.2.5 Methods for Arsenic

Burton and Statham (1982) have reviewed the occurrence of arsenic in ocean waters. Arsenic can exist in four forms: arsenate, arsenite, monomethylarsonic acid (MA) and dimethylarsinic acid (DMA). Total dissolved arsenic ranges from 0.75 - 2.0

µg/L in the ocean, the majority of which is inorganic. The organic species usually account for less than 20% of the total and are generally confined to the euphotic zone. Arsenite can be anywhere from 1 to 84% of inorganic arsenic, but it is usually about 2.5%. In interstitial waters studied by Andreae (1979), no methylated arsenicals were detected, but arsenite was. Arsenate and total arsenic concentrations were generally lower in pore waters than in the overlying seawater.

Arsine generation is the most popular technique for the separation of arsenic from environmental materials prior to quantification, usually by AAS (Brooks et al., 1981a). Godden and Thomerson (1980) have reviewed the subject of the generation of covalent hydrides in atomic absorption spectrometry. The use of a cold trap to condense arsine and organoarsine compounds purged from a sample has greatly increased the sensitivity and precision of the technique. The most sensitive detection system for arsine is a quartz cuvette burner in the light path of an atomic absorption spectrometer.

The method of Andreae (1977) is the most sensitive method available for determining arsenic species in seawater. It has been applied with meaningful results to seawater, marine algae (Andreae, 1978), and sediment interstitial water (Andreae, 1979). The latest modified version of this method is in Methods of Seawater Analysis, (Andreae, 1983.) The arsenic species are reduced to the corresponding arsines by sodium borohydride. At a pH of 6.0 to 6.5, only arsenite is converted to arsine. To reduce arsenate and the methyl arsenicals, the sample must be strongly acidified with 6N HCl. The arsines are purged from the solution with helium and collected in a liquid nitrogen cold trap filled with gas chromatographic packing. The trap is then heated electrically and the arsines elute in the sequence of their boiling points. The arsines are combusted in a quartz cuvette burner in the light path of an AAS. Alternatively, in place of the cold trap and AAS detector, the arsines can be sensitively determined by gas chromatography with electron capture detection. The determination of all four species thus requires two samples of seawater. Arsenate is determined by difference. The time required for the complete analysis is about 30 minutes. Sample size is 10 - 100 mL, depending on the concentration to be measured. The detection limit is low - about 0.3 ng/L; precision at the 70 ng/L level is 4.5% using peak integration. Interferences are minimal or absent, but the method of standard addition is preferred for calibration. Contamination is not a big problem with arsenic; clean room facilities are not required for species determination. Samples can be preserved by a quick freezing technique without altering the arsenite/arsenate ratio. Andreae's method is not expensive, nor is it particularly demanding in terms of the analyst's skill.

The basic system for arsine generation, cold trapping and selective volatization of arsenic species has also been used in conjunction with other detectors. Two variations were introduced by Shaikh and Tallman (1978) and Braman et al. (1977). Shaikh and Tallman showed that the volatized arsines can be swept directly through the sample port of a graphite furnace for detection. The detection limits for inorganic arsenic, MA, and DMA are 20, 300 and 200 ng/L, respectively using 50 mL samples. The detection system employed by Braman et al. is DC plasma discharge atomic emission spectrometry, which consists of a simple DC arc discharge cell and a scanning monochromator. The limits of detection using 50 mL samples range from 4 to 20 ng/L. Modifications which improve this method were reported by Crecelius (1978).

Alternate methods exist for determining arsenic species in water based on GF - AAS, but these are much less sensitive since the limit of detection for direct injection of an arsenic solution into a graphite furnace is about 2 μg/L. The two main techniques are:

1) separating the organic and inorganic species by ion exchange chromatography, and

2) separating and isolating the inorganic species by solvent extraction.

Iverson et al. (1979, 1980) produced an ion exchange/ GF-AAS method for determining As (III), (V), MA and DMA in sediments and interstitial waters which has a detection limit of about 2 μg/L for each species. Pacey and Ford (1981) developed a more complicated method which utilizes both anion and cation exchangers to achieve the following detection limits (μg/L): DMA .02, MA 2.0, As (V) 0.4 and total As 4.0. Lower detection limits were made possible for some species by using the ion exchange column as a preconcentrator. Aggett and Kadwani (1983) provided a simpler procedure for separating the four species of arsenic using anion exchange chromatography, which they then applied to samples of interstitial water and aquatic plants.

The solvent extraction approach is exemplified by the methods of Chakraborti et al. (1980b), Subramanian and Meranger (1981) and Puttemans and Massart (1982). These three solvent extraction methods could presumably be applied to determining arsenic in seawater and in solution matrixes resulting from acid digestion of sediments and organisms, when preconcentration and separation of arsenic from interferring ions is required. Chakraborti et al. achieved a detection limit of 6 ng/L for natural water samples by a two step extraction procedure which preconcentrates arsenic 250 times. Arsenite is extracted into ammonium sec-butyl dithiophosphate (ABDP) in hexane and back extracted into bromine water. A reducing agent ($HSO_3^-/S_2O_3^{2-}$) is then added to the re-extracted sample containing As(V) and the extraction procedure is repeated.

Subramanian and Meranger studied solution conditions and other parameters affecting the APDC - MIBK extraction system for the determination of As (III), As (V), Sb (III), Sb (V), Se (IV) and Se (VI) in water. The limits of detection (µg/L) for As, Sb and Se are .07, 0.1 and 0.3 when the ratio of aqueous to organic phase is 10. In this method the organic phase is analysed directly by GF - AAS. Puttemans and Massart developed two solvent extraction procedures for determining arsenic (III) and (V) in water samples. Arsenic (III) is extracted from acidic media with either APDC or diethyldithiophosphoric acid (HDEDTP) in chloroform and back extracted with a solution of cuprous chloride. The concentration of cuprous ion is critical to the efficiency of the back extraction. Quantitative recoveries were obtained for both extraction procedures.

Clearly the hydride generation/cold trapping/selective volatization method of Andreae (1983) is the method of choice for arsenic in interstitial waters. It has the lowest detection limits and the ability to measure all four species of arsenic. It requires a small sample, and it has been extensively applied to seawater and interstitial water. The apparatus is relatively inexpensive. The alternatives are GF-AAS combined with ion exchange or solvent extraction. The ion exchange methods are less sensitive, more time consuming and they have not been as extensively applied to seawater or interstial waters. The solvent extraction methods are less sensitive and more time consuming; they can only determine the two inorganic forms of arsenic and they have not been as extensively applied to seawater or interstitial water.

2.3 Sediments

2.3.1 Introduction

The analysis of sediment samples by AAS or ICP-AES requires some form of chemical attack - either complete dissolution or a method which liberates trace metals from the less resistant minerals such as clays or hydrolysates. Complete dissolution for the determination of major elements (i.e. those occurring at concentrations > 1%) is best accomplished by fusion with lithium metaborate according to Butler (1975). For trace elements (< 0.1%), hydrofluoric acid dissolution is preferred since it results in solutions that are as concentrated as possible without being too viscous. Minor elements (0.1 - 1. %) can be determined by either method, depending on the concentration of the element in the sample. Butler outlined the procedures for both methods and recommended conditions, such as flame and wavelength, for the determination by AAS of major, minor and trace elements in geochemical samples. Butler also described interferences, gave measures to mediate them, and provided a number of references for each element.

Thompson and Wood (1980) recommended that the fusion methods be avoided if possible, citing a variety of reasons. Fusions are inconvenient for large batches of samples, and the high concentrations of alkali metals introduced into the sample adversely affects viscosity, background absorption, precision, blank, and detection limit.

Thompson and Wood (1980) gave procedures for five different acid digestion methods for rocks, soils and stream sediments, and discussed the applicability of each. Nitric acid attack is designed for the digestion of clay minerals. Other rock-forming minerals are also digested (feldspar, olivine) or left intact (pyroxene, biotite, amphibole and quartz). Nitric-perchloric acid attack is more powerful - the digestion of pyroxenes, biotite, limonite and some amphiboles is almost complete, while rutile, chromite, cassiterite, zircon, beryl and barite mostly resist the attack. Hydrofluoric-nitric-perchloric acid attack will completely digest most constituents of rocks, soils and sediments. However, some minerals will partly or completely resist the attack (e.g. barite, chromite, cassiterite, tourmaline, kyanite, some spinels and magnetites, rutile, zircon and wolframite). Digestion by hydrofluoric acid is accompanied by the evolution of gaseous silicon tetrafluorides. As the evaporation proceeds, most of the metal fluorides are converted to sulfates and perchlorates which can be redissolved by hydrochloric acid. Sometimes boric acid is added to the digested sample to dissolve the precipitated metal fluorides. If the sample contains oil or bitumen, it must first be charred to reduce the danger of explosion. For the determination of silicon, either the hydrofluoric-boric acid attack or the lithium metaborate fusion attack is used. In the former, the digestion is carried out in a closed polypropylene bottle, and boric acid is added to dissolve precipitated fluorides. Other elements can be determined in the same solution. The latter method is useful when silicon determinations are required on samples which are not completely dissolved by the hydrofluoric-boric acid attack.

De Groot and Zschuppe (1981), in a survey of methods of analysis for heavy metals in marine sediments distinguished four types of digestion methods. These are:

i) total attack (with HF in combination with other strong concentrated acids),

ii) strong attack (with some combination of concentrated acids: HCl, HNO_3, $HClO_4$, and H_2SO_4),

iii) moderate attack (e.g., with $Na_2S_2O_4$, 2% + sodium citrate, 20%), and

iv) weak attack (e.g., ammonium acetate, 1M).

Some workers believe that it is unnecessary to obtain full digestion of all sedimentary components, including metals contained in alumino-silicate lattice minerals, since pollution effects usually occur on the surfaces of sediment particles and in allochthonous particles (Forstner and Wittman, 1981). For this reason and due to the unpopularity of HF, strong attack procedures are widely employed. Moderate and weak attack (also called partial extraction) methods are usually aimed at the authigenic or 'biogeochemically important' fraction of metals in sediments. Sequentially applied, these methods can be used to give some insight into the distribution of metals among the various chemical compounds and minerals in sediments.

Most of the analytical requirements in applied geochemistry are met by conventional flame AAS. Graphite furnace atomizers are used only when the analyte concentration is too low for flame AAS. The sampling rate for GF-AAS is 5 - 10 times slower, interference effects tend to be more severe, and more skill is required. However the flameless cold vapor atomic absorption technique for the determination of Hg is standard, and the hydride generation technique for the determination of certain elements, such as As, Ge, and Sn has become popular. In a typical exploration geochemistry laboratory, the output from an atomic absorption spectrometer is 800 to 1000 determinations per day.

2.3.1.1 Sample Storage and Preparation

The subject of sample storage and preparation for analysis has been reviewed by Forstner and Wittman (1981) and de Groot and Zsehuppe (1981). Specific recommendations are given in EPS, EPA and Inland Waters manuals (EPS, 1979; Plumb, 1981; IWD, 1979). Procedures for storing, subsampling and other preparations must ensure that a representative subsample is obtained, i.e. one that has not been altered by physical separation or chemical contamination. Sample preparation methods also depend on the kind of determination to be performed. For the determination of total metal concentrations, sediments are usually stored frozen or at low temperature (4°C) to limit biological activity. Plastic containers are usually used, although glass is also suitable. Containers should obviously be clean but special acid cleaning procedures for purifying the containers are probably not necessary. No definitive studies have been published on this aspect of sample storage. For long-term storage and before subsampling, the sediments are usually dried in the laboratory, either at room temperature or in an oven at temperatures up to 110°C. Freeze drying is sometimes preferred to air or oven drying, because it results in a powdery material; air or oven drying of clay sediments results in hard aggregates. Care must be taken to ensure that the metals to be determined are not volatilized by drying at too high temperatures. The safest and most often recommended procedure is to dry at room temperature. Mercury is sensitive to drying; losses have been observed at 60°C and as a result of freeze-drying. The procedure for mercury recommended by IWD (1979) avoids drying altogether. If all the sample is not dried, then the sample must be homogenized in the storage container before a subsample of wet sediment is withdrawn.

After drying the sample can be sieved with a 20 mesh sieve to remove stones and debris. Stainless steel sieves are sometimes used although nylon is preferred; brass sieves are a probable source of contamination for several metals. Before a sample can be subsampled for the digestion, it must be homogenized. For total metal determinations, this is usually accomplished by grinding with a mortar and pestle (usually made of agate) until the average particle size is reduced sufficiently for representative subsampling. For example, when a 100 mg quantity is to be digested in a Teflon bomb, grinding to 270 mesh or finer is recommended (IWD, 1979). For 1-2 g quantities, a 100 mesh powder may be acceptable (EPS, 1979). When the sample is to be analysed wet (e.g. for mercury) homogenization can be effected with a sturdy glass or plastic rod or steel spatula; or by kneading the sample in a plastic bag. Since metal concentrations in sediments are expressed on a dry weight basis, a separate subsample must be taken for determining water content.

Grinding increases the surface area and promotes more rapid dissolution; however grinding may adversely affect precision and comparability when a strong attack method is used because the extent of leaching depends on particle size (Agemian and Chau, 1976). It may be preferable to sieve the disaggregated sediments (100 mesh) in preparation for a strong acid leach. Forstner and Wittman (1981) have presented an argument for making determinations on the silt-clay (< 63 μm) or the pellitic (< 2 μm) fraction. The reason for doing this is to remove the often dominating influence of grain size on trace metal distribution, although this can also be done mathematically if the grain size distribution is known.

Thomson et al. (1980) recommended that sediments be extracted as soon as possible after collection if moderate or weak attack procedures are to be applied. Obviously, drying or grinding could introduce serious errors in the determination of exchangable metals or metal speciation.

2.3.2 Methods for Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

2.3.2.1 Methods for Determining Total Metal Concentrations

a) AAS Methods

The total (acid) attack - AAS methods commonly used today for determining major, minor and trace elements in aquatic sediments were developed about ten years ago. There are two principal methods, that of Agemian and Chau (1975a) designed for lake and stream sediments, and that of Rantala and Loring (1975), designed for silicate rocks and marine sediments. Rantala and Loring's (1975) digestion method is similar to Buckley and Cranston's (1971), although their instrumental methods are different. Rantala and Loring's method is described in more detail by Loring and Rantala (1977) and this was adopted by Walton (1978) in Methods for Sampling and Analysis of Marine Sediments and Dredged Materials.

The two principal methods are very similar - both employ Teflon bombs to digest small to large (0.1 -1 g) samples of dry sediment with a mixture of concentrated acids including HF. The determination of metal concentrations is accomplished by flame AAS. Agemian and Chau's method utilizes perchloric and nitric acid which is more effective in mineralizing organic matter than aqua regia used by Rantala and Loring. The more rigorous nitric-perchloric acid oxidation is appropriate for lake sediments which sometimes contain very large quantities of organic matter. The hydrofluoric -aqua regia digestion of marine sediments sometimes leaves a black residue which is generally less than 3%, when 1 gram samples are digested, and its elemental contribution is negligible, according to Rantala and Loring. Agemian and Chau stated that the hydrofluoric-nitric-perchloric digestion effects complete dissolution of the sample. Agemian and Chau's digestion takes longer - 3.5 hours compared to only one hour for the method of Rantala and Loring.

The methods also differ in the way in which samples are calibrated against standards and in how interferences are handled. The approach of Buckley and Cranston (1971) is perhaps the most rigorous, since it attempts to match the matrix of standards and samples. The assumption inherent in this method is that if elements are combined in a single standard solution in the same proportions as present in the sample, then all mutual interferences will be compensated and no need will exist for addition of excess salts or acids to standards or sample solutions. In practice such matching of standards and samples is difficult if not impossible to achieve. Rantala and Loring took the other approach - that of adding appropriate matrix modifiers such as KCl to samples and standards to overcome the interferences in lieu of matching standard with sample matrix. This is also the method adopted by Agemian and Chau, but the salts added to control particular interferences differ. Standards, whether single element, combined or matrix matched, were prepared in the same acid decomposition blank matrix as the samples.

Rantala and Loring demonstrated the accuracy and precision of their procedure by analyzing 21 elements in seven USGS rock standards. Close agreement with accepted values was found for most elements. Two exceptions were noted -

chromium and zinc could not be accurately determined in two standard rocks due to incomplete dissolution of chromite. Also, nickel values were low in two rocks probably due to interelement interference. Coefficients of variation are <5% for major and minor elements, and for trace elements precisions are generally <10%. Agemian and Chau achieved quantitative recoveries for all 20 elements by spiking sediment samples at three different levels. Additional data on precision and recovery for Agemian and Chau's method are given in the Analytical Methods Manual (IWD, 1979). Rantala and Loring's reported precisions are similar.

A procedure for digesting larger quantities (1 - 5 g) of sediment using HF + HNO_3 + $HClO_4$ in Teflon beakers is also given in the Analytical Methods Manual. Digesting larger samples will result in lower limits of detection, but silicon and perhaps chromium will be lost from the open beakers during the digestion. Limit of detection data are given for the two digestion procedures, 0.1 g sample in Teflon bomb and 1 g sample in Teflon beaker, in Table III-2.7. Data are from the Analytical Methods Manual; there is no explanation of how they were determined. The elements determined by Rantala and Loring include those given in Table III-2.7 (except molybdenum) plus Sr, Ti, Na and Rb. Data from Rantala and Loring (1975, Table III) show their quantitation limit to be: 5 µg/g Ba; 0.5 µg/g Be; 12 µg/g Ni, 12 µg/g Pb; and 15 µg/g V from the digestion of 1 gram samples. Table III-2.8 contains concentration ranges for trace metals in Beaufort Sea sediments (Thomas et al., 1982) for comparison.

Assuming that the limit of quantification is approximately three times the limit of detection, it can be inferred from these data that the following metals cannot be adequately determined in Beaufort Sea sediments at the lower end of the concentration range by digesting a 0.1 g sample in a Teflon bomb and measuring the concentrations by flame AAS: Ba, Be, Cd, Cu, Cr, Pb, Ni, V and Zn. Of these metals, Cd, Pb and possibly Be may not be determined by digesting a 1 g sample in a Teflon bomb or beaker.

b) GF-AAS Methods

The use of graphite furnace atomizers permits the accurate determination of many metals which occur at concentrations far below the limits of quantification for flame AAS methods. Matrix interference effects are fairly common and the method of standard additions is often employed.

Owens and Gladney (1976) accurately determined Ba, Be, Cd, Co, Cr, Cu, Mn,

TABLE III-2.7

DETECTION LIMITS (μg/g) OF FLAME AAS METHODS FOR SEDIMENTS
From Analytical Methods Manual, (IWD, 1979)

Metal	Teflon Bomb[1]	Teflon Beaker[2]
Al	500	1 - 5000
Ba	100	
Be	10	
Cd	10	1 - 200
Ca	10	
Cr	10	
Co	10	1 - 500
Cu	10	1 - 500
Fe	50	5 - 500
Pb	50	5 - 2000
Li	5	
Mg	3	
Mn	10	1 - 300
Mo	50	5 - 4000
Ni	10	1 - 500
K	100	
Si	500	
Na	100	
V	50	1 - 15000
Zn	10	1 - 100

[1] Detection Limit (μg/g).
100 mg sample, 1000 x dilution

[2] Analytical Range (μg/g).
1 g sample, 100 x dilution

TABLE III-2.8

RANGE OF TOTAL METAL CONCENTRATIONS (µg/g) FOR BEAUFORT SEA SEDIMENTS

(from Thomas et al., 1982)

Metal	Range	
As	2	- 17
Ba	10	- 660
Be	0.81	- 1.2
Cd	0.011	- 2.2
Cr	3.1	- 250
Cu	1.9	- 450
Hg	0.007	- 0.34
Ni	5.9	- 120
Pb	1.6	- 100
V	10	- 200
Zn	13	- 900

Mo, Pb, V and Zn in NBS coal fly ash by GF-AAS. The sample (0.5 g) was fused for 15 minutes with 1.0 g $LiBO_2$ in a platinum crucible at 1000-1100°C, the melt permitted to cool and then dissolved in 50 mL of 10% HNO_3. The fusion/dissolution procedure took about 30 minutes and did not cause any of the above metals to be lost through volatization. The high salt matrix reportedly did not interfere with the GF-AAS determination; standards were made up in the $LiBO_2$/nitric acid blank matrix. Also, blanks were reasonably low for all of the elements determined.

Sakata and Shimoda (1982) reported a method for determining Pb and Cd in sediments which gives detection limits of 1.0 and 0.04 µg/g, respectively. The digestion method of Agemian and Chau, recommended by Sakata and Shimoda, effectively dissolved the silica matrix, but did not completely decompose the organic matter present. Several major elements caused serious interferences in the determination of Cd and Pb by graphite furnace AAS, but the interferences were overcome by the addition of 1% $(NH_4)_2SO_4$ and 1% $(NH_4)_3PO_4$, respectively. The method of standard additions was not required. Hsu and Locke (1983) proposed a modification of the Agemian and Chau Teflon bomb digestion in which the oxidizing power is increased by raising the proportion of perchloric acid and the temperature to 150 C. The method gave precise and accurate values for Cd and Pb for a variety of standard and natural sediments. Rantala and Loring (1980) developed a method specifically for cadmium in marine sediments which gave accurate and precise results for standard sediments and rocks in which cadmium is present at very low levels. Relatively large (1 g) samples are digested in Teflon bombs according to the method of Rantala and Loring (1975) and Cd is determined directly by GF-AAS using standard uncoated graphite tubes. Calibration solutions were prepared with cadmium metal in the acid decomposition blank matrix.

Sturgeon et al. (1982) have tried a different approach to the interference problem - a stabilized temperature (L'Vov) platform graphite furnace. They obtained accurate results for Cd, Pb, Cu, Ni, Co, Be and Cr in total acid attack solutions of the NRC marine sediment reference materials, MESS-1 and BCSS-1. The digestion involved treatment with HF, HNO_3, $HClO_4$ and HCl in two successive extractions, carried out in a Teflon beaker. They also showed that the technique is virtually interference-free; the sediment solutions are calibrated against simple aqueous standards and matrix modifiers are not used. Detection limits, for the method, calculated as three times the standard deviation of the blank in µg/g, are: 0.006 for Cd, 0.15 for Pb, 0.18 for Cu, <0.15 for Ni, <0.06 for Co, <0.009 for Be and <0.009 for Cr. These are much lower than those obtained by flame AAS and would permit determinations with good precision at the lowest levels of these metals occurring in

Beaufort Sea sediments.

Sturgeon et al. (1982) also evaluted the technique of chelation-extraction to separate the analytes from interfering elements in the sediment solutions. Relative to the direct method, the chelation-extraction technique was more time consuming and less precise. Sanzolone et al. (1979) extracted sediment solutions with MIBK and sodium diethyldithiocarbamate after masking iron and aluminum with ammonium fluoride. The manganese interference was removed by taking advantage of the instability of that metal in the extracted solution. The lower limits of determination of Co, Ni and Cu were about 5 ppm by flame AAS.

c) ICP-AES Methods

The use of inductively coupled plasma atomic emission spectrometry in the analysis of geological materials is increasing. The main advantages are simultaneous multi-element capability, superior sensitivity compared to flame AAS and relative freedom from matrix interference effects.

Three reports of complete methods that are suitable for marine sediments were found, viz. McLaren et al. (1981), Floyd et al. (1980a) and McQuaker et al. (1979a, b). The three methods are similar in performance, but there are differences in digestion methods and instrumental techniques applied. In the analysis of sediment solutions by ICP, it is necessary to avoid high concentrations of dissolved solids which disturb nebulizer function, while it is desirable to dilute the original sample as little as possible for maximum sensitivity. The digestion methods of McLaren et al. (1979a,b) uses hydrofluoric, nitric and perchloric acids. It is carried out in a Teflon bomb immersed in boiling water; after 2 hours the contents are evaporated to dryness on a hotplate. The residues are then dissolved in 1 N hydrochloric acid, resulting in a solution that is less than 1% in dissolved solids. The McQuaker et al. (1979a, b) digestion is done in an open Teflon beaker using $HClO_4$ + HNO_3 (with or without HF); the solution that is presented to the ICP is 3.5% in acid. In Floyd et al.'s method, the sample is fused with sodium hydroxide in a platinum crucible, and the melt is dissolved with hydrochloric acid resulting in a sodium chloride concentration of 2.9%.

The investigation of spectroscopic interferences is much more important in the case of sediment solutions than in the case of seawater extracts. Interferences due to ionization suppression and refractory compound formation are considered to be negligible for the inductively coupled argon plasma. Spectral interferences were investigated by analysing wavelength intensity profiles, and analytical wavelengths were judiciously chosen to minimize spectral overlaps. Approaches to correcting for spectral interferences varied depending on the capabilities of the instruments employed. The ICP spectrometer used by Floyd consisted of a computer-controlled scanning monochromator (Floyd et al., 1980b). As each sample is run, the computer steps the monochromator to the appropriate analyte lines and background wavelengths, measures the intensities and calculates the concentrations of each element using the appropriate analytical calibration curves. The instrument used by McLaren also had sequential capability, and background intensities were measured at .02 - .03 nm above and below the analyte wavelengths. The instrument used by McQuaker et al. (1979a,b) is a polychromator, so interference corrections were obtained by comparing sample solutions with reference solutions for which inter-element effects were absent. All three methods used multi-element calibration solutions made in the same acid digestion blank as the samples. No attempt was made to matrix match the calibration to the sample solutions. Nebulization effects were found to be negligible for the perchloric acid matrix.

The limit of detection (LOD) for the three methods, shown in Table III-2.9, are similar, despite differences in sample matrix composition and dilution factor. The detection limits reported correspond to the concentration of analyte required to produce a signal equal to three times the standard deviation of the blank solution. Thus they are not method LOD's, but are probably a good approximation to them in this case. Comparing LOD's for the ICP methods and the flame AAS methods, the ICP numbers are generally lower. The improvements for Ba and Be are 100X and 50X, respectively while those for other metals are generally less than 10X. The ICP LOD's are generally higher than those obtained by the graphite furnace AAS method of Sturgeon et al. (1982). For Be, Cd, Cr, Co, Ni and Pb the factors range from 3 to 50. The copper LOD's are the same. It is clear from Tables III-2.8 and III-2.9 that the sensitivity of the ICP methods are adequate for metals in Beaufort Sea sediments except for As, Cd and Pb at the lowest concentrations.

Accuracy and precision of the three ICP methods was demonstrated by analysing reference materials. Floyd et al. (1980a) determined 26 elements in NBS river sediments and 33 elements in four USGS rocks. Low values were obtained for chromium in two of the rocks. McLaren et al. (1981) determined 14 elements in NRC marine sediments (BCSS-1 and MESS-1). Of the trace elements, Be, Co, Cu, Mn, Ni, Pb, V and Zn were determined accurately, while As, Mo and Cd were below the limit of quantitative determination. Chromium and titanium values were too low because

TABLE III-2.9

DETECTION LIMITS (μg/g) OF ICP METHODS FOR SEDIMENTS

	Method 1	Method 2	Method 3
Al	1	1.4	
As	5	3.9	5
Ba	0.25	0.03	
Be	0.03	0.02	0.05
Ca	0.05	0.8	
Cd	0.5	0.2	0.2
Cr	0.5	0.4	
Co	1	2.9	0.5
Cu	0.2	0.3	0.1
Fe	0.25	0.4	
Mg	0.02	3.1	
Mn	0.05	0.06	0.05
Mo	3	0.4	1.5
Ni	1	1.7	0.6
Pb	10	3.7	3
Sb	15	5	
Se	35	5.2	
Si		1.3	
Sn	5	2.9	
Ti	0.2	0.2	
V	0.1	0.2	0.3
Zn	0.1	0.3	0.1

Method 1 - Floyd et al. (1980)
Method 2 - McQuaker et al. (1979a, b)
Method 3 - McLaren et al. (1981)

of incomplete dissolution. McQuaker et al. (1979a) determined 20 elements in reference soil samples; the elements included all of the metals of interest except mercury. The results generally compared favorably with the reference values (McQuaker et al., 1979b). Low recoveries of Al, Fe and Mn were attributed to extraction inefficiencies associated with digestion in open vessels.

Burman et al. (1978) reported a method for preparing rock samples for ICP analysis consisting of 1+1 fusion of the sample with $LiBO_2$. This procedure results in a relatively low concentration of dissolved salts compared to the usual practice of using flux to sample ratios of 7-10. The reliability of the procedure was shown by analysing standard rocks. Apparently, if the procedure results in incomplete destruction of silicates, the large complexes formed are subsequently decomposed in the plasma. Brzezinska et al. (1984) evaluated seven different digestion procedures commonly used for biological and geological samples for suitability to ICP analysis. They preferred total attack in a Teflon bomb to fusion techniques. The advantages of Teflon bomb digestion are that contamination is avoided, decomposition is speeded up, and the volume of acids used is minimized. They also recommended two procedures for geological materials and applied one of them to the NRC marine sediment reference materials. Accurate concentrations were obtained for 16 elements including Zn, Ni, Pb, Cr and V, although As, Cd and Cu were below the limit of quantitative determination.

Burman and Bostrom (1979) have compared capacitively coupled microwave plasma (MWP) and inductively coupled plasma excitation (ICP) sources for the analysis of geological materials. Analyses by MWP require careful matching of sample and calibration solutions to get satisfactory results. Also, a large amount of ionization buffer is needed. The freedom from matrix effects makes it easy to prepare sample and calibration solutions for ICP. Also, nebulizer function is more sensitive to disturbances with ICP. The low detection limits, extended dynamic concentration range and relative freedom from matrix interference problems make ICP superior to MWP in the analysis of geological samples.

d) Summary and Conclusions

A variety of methods have been presented for determining total trace metal concentrations in sediments. All of the methods consist of a dissolution procedure and a procedure for quantifying the metals in solution. The methods for bringing the samples into solution are compared in Table III-2.10. There are two critical factors, that the elements of interest are completely dissolved and that they are not lost by volatilization or fixation to solid components of the system. For the methods in question, incomplete dissolution is sometimes a problem for some elements in some sample matrixes, while losses are unlikely for the elements of interest. Fusion methods are perhaps less likely to give incomplete dissolution than acid dissolution methods but they are inconvenient for most laboratories to perform and they result in a more viscous solution and higher blanks. Of the acid dissolution methods, those involving evaporation are particularly attractive because they result in a final solution that is 1M in HCl, which is ideal for instrumental quantification. The method of McLaren et al. (1981) has the additional advantage that the digestion is performed in a Teflon bomb.

The instrumental methods are compared in Table III-2.11. When detection limits of flame AAS are inadequate, it will be necessary to resort to the graphite furnace. When the detection limits are adequate the flame will be preferred since it is more rapid and less subject to interference. ICP has a number of advantages over flame AAS, but some elements will still be below the limit of quantitative determination. A useful approach would be to use ICP and AAS in combination when possible. Since the two instruments are subject to different kinds of interferences, they could be used together to check the accuracy of the determinations.

Cost of analysis is usually an important factor in choosing a method. In order to make a rough assessment of the relative cost of sediment analyses using the three instrumental techniques, current prices from four commercial laboratories in Vancouver and Victoria are summarized in Table III-2.12. The costs of analysis by flame and graphite furnace are similar, with graphite furnace usually being a little more expensive. When a large number of elements are to be determined, ICP is considerably cheaper. The crossover point occurs somewhere between one and eight elements. The wide range of per sample prices suggests that there may be a corresponding range in quality of the analyses. It is therefore a good idea, in selecting a firm to do chemical analyses, to scrutinize the analytical methods and the quality control programs they employ. One can also include spiked and reference samples and replicates among the regular samples to be analysed (Macdonald and Nelson, 1984).

TABLE III-2.10
COMPARISON OF TOTAL ATTACK METHODS FOR SEDIMENT DIGESTION

SUMMARY OF METHOD	ADVANTAGES	DISADVANTAGES	REFERENCE FOR METHOD
a) Acid digestion in Teflon bomb			
HF + Aqua regia	- higher temperature possible with Teflon bomb than with open vessel. - Teflon bomb digestion requires smaller quantities of acids. - Teflon bomb reduces the risk of contamination. - accuracy evaluated with U.S.G.S. standard rocks.	- low recoveries of Cr, Zn obtained for some rocks.	Buckley & Cranston (1971) Rantala and Loring (1975)
HF + $HClO_4$ + HNO_3	- more effective than HF + aqua regia for destroying organic matter. - spike recoveries verified.	- accuracies not verified by analysing standard reference sediments (except for Cd and Pb).	Agemian and Chau (1976) Hsu and Locke (1983)
HF + $HClO_4$ + HNO_3 digestion in Teflon bomb, evaporation to dryness, redissolution in 1 M HCl	- gets rid of HF and other strong acids by evaporation, eliminating need for boric acid and resulting in low salt/acid matrix suitable for ICAP-AES. - accuracies verified by analysing standard reference sediments.	- evaporation step takes longer. - possible volatilization losses. - low values for Cr, Ti in NRC sediments attributed to incomplete dissolution of certain minerals.	McLaren et al. (1981)
b) Acid digestion in Teflon beaker			
One treatment with HF + $HClO_4$ + HNO_3	- digestion of larger samples possible in a beaker than in a bomb, resulting in lower detection limits. - spike recoveries verified.	- possible volatilization losses, eg. CrO_2Cl_2 boils at 116 °C.	I.W.D. (1979) Agemian and Chau (1976)
Successive treatments with HF, $HClO_4$, HNO_3 and HCl, final solution in 1 M HCl	- 2 evaporation steps to remove HF and other strong acids; boric acid not used; results in low salt/acid matrix suitable for GF-AAS. - very effective dissolution procedure. - accuracies verified by analysing standard reference sediments.	- more time consuming than single treatment.	Sturgeon et al. (1982)
c) Fusion methods			
$LiBO_2$ fusion	- accuracies verified for NBS fly ash. - fairly rapid procedure, takes about 30 minutes.	- inconvenient for batch processing. - yields higher salt content solution than acid digestions, leading to higher blanks, viscosity and matrix interferences.	Owens and Gladney (1976)
NaOH fusion	- accuracies verified for a number of standard reference materials.	-yields relatively high salt content, 2.9% NaCl. - values for Cr low for U.S.G.S. rocks. - inconvenient for batch processing.	Floyd et al. (1980)

TABLE III-2.11

COMPARISON OF INSTRUMENTAL METHODS FOR DETERMINATION OF TOTAL METAL CONCENTRATIONS IN SEDIMENTS

	FLAME - AAS	GF - AAS	ICP - AES
References	Agemian and Chau (1975) Rantala and Loring (1975) Buckley and Cranston (1971)	Caravajal et al. (1983) Sturgeon et al. (1982)	McLaren et al. (1981) Floyd et al. (1980a) McQuaker et al. (1979)
Sensitivity and Detection Limits	Least sensitive. Detection limits are adequate for major and minor and most trace elements.	Most sensitive. Detection limits are adequate for all trace elements of interest.	Intermediate in sensitivity. Detection limits are adequate for most trace elements of interest (Cd and Pb are exceptions).
Interferences	Chemical and ionization interferences exist and are usually handled with matrix modifiers. Spectral interferences are uncommon. Standards are made in acid decomposition blank.	Matrix effects are more prevalent than with flame. Method of standard additions is often necessary. Background correction is usually required. Matrix modifiers and pyrolytically coated tubes improve performance for some elements. L'vov platform eliminates the need for standard additions.	Chemical and ionization interferences are negligible. Spectral intereferences require correction, which is very effective. The approach depends on the capabilities of the instrument used, usually involves spectral analysis and computerized calculations. Matrix matching and matrix modifiers are unnecessary.
Speed and Convenience	Individual elements are determined one at a time in a batch of samples. Takes less than 1 minute per sample.	Slower than flame, especially if method of standard additions is used. GF-AAS methods require more skill than flame methods.	Simultaneous multi-element capability. Much faster than flame AAS when a large number of elements are determined. ICP instrument requires more skill and training to operate than AAS.

TABLE III-2.12

COMPARISON OF COSTS FOR TRACE ELEMENT ANALYSIS OF SEDIMENTS USING AAS AND ICP - AES

	AAS				ICP		
Metals	**Firm 1**	**Firm 2**	**Firm 3**	**Firm 4**	**Firm 1**	**Firm 2**	**Firm 3**
Fe, Al, Ba, Be, Cd, Cr, Cu, Ni, Pb and V (Aqua regia digestion)	$35/sample + $4/element(F) $8/element(GF)	$10/sample + $5/element(F) $8/element(GF)	$10-20/element (F or GF)	$25-30/sample + $5 - 7/element (F or GF)	$45/sample (30 elements included)	For Geochemical samples: $8/sample (13 elements) $13/sample (24 elements)	$25/sample (31 elements)
Hg (CV-AAS)	$25	$10	$21	$25			
As (Hydride Generation)	$25	$10	$18				$25 (includes As, Se, Bi, Sb and Sn)

2.3.2.2 Strong Acid Attack Methods

Several authors have reported investigations of the efficacy of strong (acid) attack methods for determining trace metals in sediments by AAS. Agemian and Chau (1976) compared the extraction of metals from a Lake Ontario sediment using total attack with HF + HNO_3 + $HClO_4$ (6 + 4 + 1) in a Teflon Bomb and three strong attack methods - HNO_3, aqua regia, and HNO_3 + $HClO_4$ (1 + 1) carried out in Teflon beakers. On the basis of what is known about the strength of these acids, the expected ranking of extraction efficiency of the methods would be, in decreasing order: HF + $HClO_4$ + HNO_3; $HClO_4$ + HNO_3; HNO_3 + HCl; and HNO_3. The percentages of metals leached by the three strong attack methods (relative to that obtained by total attack) ranges from about 15% (Co, Ni, Mn and Cd) to about 90% (Cu, Fe and Zn). The three strong attack methods gave very similar results for each metal with the exception of $HClO_4$ + HNO_3 for Cr which was low, perhaps because CrO_2Cl_2 was volatized. It is apparent that the strong attack methods may give substantially lower values than those obtained from total attack for some metals and sediments. Agemian and Chau pointed out that extracting efficiency for the strong acids depends on the size of the sediment particles and on the temperature of the digestion (extraction increases with increasing temperature).

Sinex _et_ _al_. (1980) evaluated a method involving HNO_3 + HCl (9 + 1) digestion with flame AAS determination by comparison with a reference method, lithium metaborate fusion with direct current plasma emission spectrometry. The two methods were applied to two sediments - NBS river sediment standard (NBS - 1645) and a sample of mud from Chesapeake Bay. The strong acid attack methods gave values which agreed with the certified ones for the NBS sediment standard, but when the two analytical methods were applied to the Chesapeake Bay samples, the strong attack method's results were lower for chromium. This was explained as incomplete dissolution of chromium-rich minerals such as chromite. The 9 + 1 mixture of nitric and hydrochloric acid is comparable to aqua regia in completeness of extraction and has the advantage of a lower blank. Boiling increased trace metal recoveries by up to 12% compared to shaking at 80 C. The completeness of extraction was insensitive to changes in the sediment-to-acid ratio in the range 1 - 5 g per 100 mL. An extraction time of 2 hours was found to be sufficient, but 4 hours is recommended.

Krishnamurty et al (1976) proposed a strong attack procedure for sediments utilizing nitric acid and hydrogen proxide (10 + 3) that was claimed to be safe and effective. Preliminary oxidation of the sample with nitric acid eliminates the danger of explosion. The reagents are available commercially with very low trace metal contamination, resulting in low blanks. The accuracy of the proposed method was determined by comparison with three other strong attack methods. The nitric acid - hydrogen peroxide method generally gave values that agreed with those obtained by the other methods. Anderson (1974) evaluated both $H_2SO_4 + HNO_3$ and $HNO_3 + HCl$ digestion procedures and found that the former caused a considerable loss of lead (11-35%) during filtration, whereas the latter is an excellent extraction procedure for the determination of metals not bound in sediment minerals.

Caravajal et al. (1983) developed and tested a method for the determination of 23 elements (including arsenic) in river sediments by graphite furnace AAS. The method involves successive digestions with nitric and hydrochloric acids. Pyrolytically coated graphite tubes improved the sensitivity for some metals, and the method of standard additions was used for all metals. Special graphite furnace operating conditions, including the use of matrix modifiers, were developed to ameliorate the severe matrix interferences encountered for Cu, Pb, As and Cd. Accuracy was assessed in two ways - by analysing a standard river sediment (NBS 1645) and by using a reference method, lithium metaborate fusion - energy dispersive x-ray fluorescence spectrometry, to analyse Arkansas river sediments. Good accuracy for Mn, Cu, V, Cr, Ni, As, Pb, Zn and Mo was demonstrated for the acid extraction-graphite furnace AAS method, even in the presence of matrix interferences. The nitric-hydrochloric extraction did not completely dissolve all of the Fe, Cd, Co, Sb and the alkali and alkaline earth metals, however.

The strong attack methods are summarized and compared in Table III-2.13. The work of Agemian and Chau (1976) showed that different strong attack procedures can yield results that are quite comparable. Strong attack methods can be used to give a close approximation to the total value for certain elements. Sulfuric acid should perhaps be avoided since it may create problems with graphite furnace analysis and certain metal sulfates are insoluble. Volatilization of chromium is possible using perchloric acid. Though not usual, there is no reason why a strong attack should not be performed in a Teflon bomb.

TABLE III-2.13

COMPARISON OF STRONG ACID ATTACK METHODS FOR SEDIMENTS

METHOD SUMMARY	EXTRACTION EFFICIENCY	COMMENTS	METHOD REFERENCES
3 Methods Compared: HNO_3, aqua regia and $HClO_4 + HNO_3$	Extraction efficiencies were similar for the 3 methods: 70-100% for Cu, Fe, Zn, Pb 10-45% for Co, Mn, Ni, Cd, Cr.	Recoveries for Lake Ontario sediments were evaluated relative to a total method. Low recovery of Cr for $HClO_4 + HNO_3$ leach, believed due to volatization of CrO_2Cl_2.	Agemian and Chau (1976)
Successive treatments with HNO_3, $HNO_3 + HCl$, HCl	Complete extraction of Hg, Ni, Cr, Cu, Mn, As, Pb, Zn, V. Incomplete extraction of alkali and alkaline earth metals, Fe, Cd, Sb, Co.	Recoveries were evaluated for NBS river sediment and Arkansas River sediment relative to a total method.	Caravajal et al. (1983)
$HNO_3 + HCl$ (9 + 1)	Complete extraction for Cr, Mn, Ni, Cu, Zn, Pb. Incomplete for Fe, Co, Cd.	Recoveries were evaluated for NBS river sediment and Chesapeake Bay sediment relative to a total method.	Sinex et al. (1980)
$HNO_3 + H_2O_2$	Complete extraction for Fe and Mn in NBS phosphate rock and for Mn and Pb in NBS orchard leaves.	Extraction efficiency was poorly evaluated.	Krishnamurty et al. (1976)
$HNO_3 + H_2SO_4$	Incomplete extraction for Pb.	Low Pb recovery due to precipitation of $PbSO_4$.	Anderson (1974)

2.3.2.3 Moderate and Weak Acid Attack Methods

The use of intermediate and weak attack methods in pollution studies is increasing as a rapid screening method and as an indicator of biologically available metal. Chester and Voutsinou (1981) demonstrated that leaching sediment samples with cold 0.5 N HCl is a rapid, inexpensive way of initially establishing the degree to which a sediment population has been subjected to trace metal pollution from the overlying waters. The cold 0.5 N HCl leach was chosen from among a variety of techniques designed to liberate all, or part, of the non-residual (including that which originates from polluted waters) trace metals from sediments. Loring (1981) used a weak 25% acetic acid leach to make an assessment of the concentrations of metals in Eastern Canadian coastal sediments that are potentially available to the biota. Loring argued that measurement of total metal concentration in sediments is a poor means of assessing the potential or actual availability of a given element to biota. Luoma and Bryan (1978) investigated the factors controlling the availability of sediment-bound lead to Scrobicularia plana, an estuarine bivalve. The concentration of lead in the bivalve can be predicted from the Pb/Fe ratio in 1N hydrochloric acid extracts of surface sediments. This was interpreted as reflecting the partitioning of lead between different phases in the sediments, i.e. between biologically unavailable iron oxide bound lead and more available organically bound lead.

Four comparative studies of partial extraction methods illustrate the range of techniques that have been tried and the problems encountered in their application. Agemian and Chau (1977) compared 4.0 M HNO_3 - 0.7 M HCl, 0.5 M HCl, 1M hydroxylamine hydrochloride - 25% acetic acid, and .05 M EDTA as extractants of non-detrital heavy metals from sediments of the Rideau River. Malo (1977) evaluated 0.3 M HCl, pH 3 or pH 7 citrate-dithionite and 1M hydroxylamine hydrochloride - 25% acetic acid for their potential to remove surface coatings from sediment particles using 10 samples from diverse marine and fresh water environments. Luoma and Bryan (1981) examined the suitability of 10 different extraction techniques (strong, moderate and weak) for operationally defining an extractable phase of trace metals in sediments and for determining the bioavailability of sediment-bound metals. The sediments of 19 estuaries from south and west England were used to test the methods. Finally, Van Valin and Morse (1982) compared one weak, 4 intermediate and 2 strong extraction techniques using 8 different (marine) sediment types. The sequential extraction procedure of Tessier et al. (1979) was also studied.

The results of these four studies are generally consistent in that they show that varying amounts of trace metals are extracted depending upon the particular metal, the sediment type and the extraction technique used. As one would expect, the amounts of metal extracted decrease in the order: strong > intermediate > weak extraction. Two studies found that different patterns of metal release were observed for oxidizing and reducing sediments. In reducing sediments sulfides are generally the dominant substrate controlling trace metal concentrations. Luoma and Bryan found significant correlations between the amount of trace metals extracted by particular techniques and the amount of operationally defined sediment components. Their results suggest that the metals are competitively divided among available substrates. By interacting more or less selectively with the available substrates, an extracting solution will cause a redistribution of trace metal among the remaining solid substrates and the solution phase. Thus the amount of metal partitioned to a particular sediment component as determined by a selective or partial extraction technique is an operationally defined quantity.

It is noteworthy that three of these four comparative studies concluded that 0.3 - 1.0 N HCl best satisfied the criteria for a useful intermediate strength extraction technique. While Agemian and Chau (1977) determined that hydroxylamine hydrochloride and acetic acid is not satisfactory for extracting authigenic sulfides and organic complexes, Van Valin and Morse (1982) preferred 1.0 N hydroxylamine hydrochloride + 25% acetic acid to 0.5 N HCl because it exhibited higher extraction efficiencies.

It is difficult to determine what criteria were used by each set of investigators to evaluate extraction procedures, since these were not always explicitly stated. Generally the extraction methods were compared and evaluated on the basis of extraction efficiency and limitation of attack on crystalline aluminosilicate minerals. Also considered were such factors as simplicity, ability to extract all metals investigated, and freedom from operational difficulties. Ideally, a partial extraction technique should measure only metals bound to non-detrital or authigenic phases. This would result in the greatest contrast between background and anomalous samples. Luoma and Bryan studied the specificity of methods for extracting metals bound to particular sediment substrates by evaluating statistical correlations between the amounts of metals released by particular extractants and the amounts of operationally defined substrates present in the samples.

Some operational difficulties encountered in these studies are:

1) Low precision for analysis of metal concentration in sediment subsamples, which could be due to inhomogeneity of the sediment subsamples or lack of ruggedness in the extraction procedure.

2) Inability of extractant to retain released metals quantitatively in solution. This can happen when an acid extractant has insufficient strength to counteract the neutralizing effect of carbonates in sediment samples, when a weak extractant is not buffered at a sufficiently low pH, or when sulfides are present in sufficient amount to reprecipitate metals extracted from other substrates.

3) Insufficient limitation of structural damage. Concentrated acids do attack aluminosilicate minerals. Agemian and Chau also found that 4.0 N HNO_3 + 0.7 N HCl has a considerable effect on aluminosilicate crystal lattices. There is general agreement that 0.3 - 1.0 N HCl does not attack aluminosilicate minerals to an appreciable extent.

Selective (and sequential) extraction techniques may be prone to reproducibility problems. Malo states that precision compares favourably with direct metal analysis by A.A.S. However, data given by Van Valin and Morse show that there is a rather high variability associated with individual (sequential) extraction steps, which tend to cancel when all the steps are summed. Partial extraction methods are probably not very rugged, i.e. they are sensitive to minor variations in extraction procedure. Thus, intercomparability of different labs using the same method would be expected to be poorer than for the total and strong attack methods. Also, there are as yet no certified values for selective extraction of standard or reference sediments. There is however, an intrcalibration exercise being carried out by ICES evaluating and comparing 1 N HCl and 25% acetic acid extractions.

The effect of sample storage on the extraction of Cu, Zn, Fe, Mn and organic material from oxidized estuarine sediments was investigated by Thomson et al (1980). Nine different extractants were applied to separate subsamples subjected to four different storage treatments. Analysis of variance was used to test if treatments significantly affected metal extractability. The authors concluded that freezing or drying are adequate methods of storage if the sediments are to be extracted only with HCl. Only freezing is satisfactory for other types of acid extraction. If a variety of extractants is to be used, the extractions should be conducted as soon as practical after collection.

2.3.2.4 Summary

Methods for determining trace metals in sediments have been classified according to the kind of extraction methods used to bring the metals into solution. The four kinds of extractions are total, strong, moderate and weak. Examples of the total and strong extraction methods have been presented and compared in Tables III-2.10 and III-2.13. The instrumental techniques for determining metals in extraction solutions have been compared in Table III-2.11. To provide an overview, Table III-2.14 compares the main categories of extraction technique.

2.3.3 Methods for Mercury

Total mercury in sediments is determined principally by automated and manual cold vapor atomic absorption techniques. The automated techniques are continuous in the sense that the flowing sample is continuously reacted in a manifold, while the manual techniques use discrete samples. The automated methods offer some advantages in terms of speed and precision, at the expense of sensitivity, though this is not necessarily a problem for sediments or biological materials.

The non-automated methods can be differentiated by the technique used for transferring mercury vapour into the absorption cell. Mercury vapour may be displaced from the reduction flask with a purge gas or with water, passed through the cell and vented, or it may be continuously cycled through the system with a pump. Mercury vapour can also be generated by the reduction reaction in a syringe, then injected into the absorption cell. The conventional technique is to bubble a purge gas into the solution immediately after adding reducing agents to it. Alternatively, the solution is agitated after adding the reagents until partitioning of mercury atoms

TABLE III-2.14

COMPARISON OF EXTRACTION METHODS FOR METALS IN SEDIMENTS

	DEFINITION	PURPOSES	EXTRACTION EFFICIENCY	PRECISION, ACCURACY, REPRODUCIBILITY, COMPARABILITY	OPERATIONAL DIFFICULTIES
TOTAL ATTACK	Mixture of strong acids plus HF plus heat. Fusion methods, eg. $LiBO_2$ are rarely used.	To determine total metal concentration, including metals in all sediment components.	Nominally total metal concentration. Actually a few minerals can resist dissolution; incomplete recoveries for some metals possible.	Generally good precision, reproducibility and accuracy. Overall accuracy can be evaluated using S.R.M.'s. Rugged. Good comparability among different procedures.	Possible volatization losses (avoided with Teflon bomb). Highest blanks. Hydrofluoric acid not popular. Fusion methods not popular.
STRONG ATTACK	Strong acids (not including HF) plus heat, eg. conc. HNO_3.	To provide a measurement of 'strong acid extractable' metal, including metals in organic matter and precipitates but not including metals in minerals.	Depends on acids used, temperature and particle size. Clay minerals are completely dissolved, other minerals are generally not. (Metals contained in refractory minerals are usually not of interest).	Generally good precision and reproducibility. Rugged. Different strong attack procedures generally have similar extraction efficiencies. Accuracy cannot be evaluated.	Possible volatization losses. Pulverization of sample gives higher results. No certified values for strong attack procedures. No accepted standard procedure. Perchloric acid not popular.
MODERATE & WEAK ATTACK	Weak acids, (eg. 1 M HCl), reducing agents (eg. hydroxylamine), oxidizing agents (H_2O_2), complexing agents (citrate, oxalate), ion exchange (Mg^{2+}).	To provide an operationally defined measure of 'biologically available' or 'biogeochemically important' metal concentration. To give higher contrast between anomalous and background samples. To study partitioning of elements among sedimentary components.	Depends on mixture and concentration of acids/ reagents used. Depends on sample storage and extraction conditions.	Lower precision, reproducibility and comparability expected. Not rugged - sensitive to drying, freezing, air exposure, extraction time and temperature, etc. Accuracy cannot be evaluated.	Sensitive to storage and extraction conditions. Reprecipitation of metal sulfides possible. Possible inconsistency due to neutralization of of acids by carbonates.

between the sample solution and the vapour is complete; then the vapour is flushed through the absorption cell. In the stationary technique, the absorption cell and reduction flask are constructed in a way that allows rapid equilibrium partitioning of Hg throughout the system.

De Groot and Zschuppe (1981) have reviewed digestion methods commonly employed for determining mercury in marine sediments. Generally, mixtures of strong acids are used in conjunction with oxidizing agents. Many combinations of acids and oxidants have been tried. Perchloric acid is not usually used, because it is desireable to perform the digestion at a relatively low temperature to avoid possible volatization losses. Total attack methods using hydrofluoric acid are also rarely employed, which is consistent with the assumption that mercury exists primarily as mercuric ions adsorbed on fine-grained inorganic or organic particles, or as methyl mercury produced by microbial activity.

Agemian and Chau (1975c) compared three digestion methods for mercury: 1) $HCl + HNO_3$ (1 + 9); 2) $H_2SO_4 + HNO_3$ (2 + 1) and, 3) HF + aqua regia (6 + 1). In all three cases, the extracts were oxidized with potassium permanganate and potassium persulfate subsequent to acid leaching. The HF + aqua regia leach (performed in a Teflon bomb) produced higher blanks and is not necessary for complete extraction of mercury from the sediment. The $HCl + HNO_3$ method has the disadvantage that violent frothing, caused by evolution of chlorine gas, occurs on addition of $KMnO_4$. The $H_2SO_4 + HNO_3$ digestion was therefore recommended. Agemian and Chau (1976) have since improved on the sulfuric-nitric acid digestion by including a trace amount of HCl to help dissolve mercury (II) sulfide. Ure (1977) has shown that the apparent failure of the sulfuric-nitric acid digestion to dissolve cinnabar (HgS) can probably be explained as an interference in the $SnCl_2$ reduction of Hg (II) caused by Fe (III), formed during the wet-oxidation of FeS which is likely to occur in relatively large amounts in sulfidic soil or sediment samples.

After wet oxidative digestion, hydroxylamine is sometimes added to reduce precipitated MnO_2, which might otherwise sequester Hg (II) by coprecipitation, and to reduce excess oxidant. The reducing agent should be strong enough to reduce the excess of oxidant, but not to reduce the Hg (II). Agemian and Chau (1975a) studied a number of different reducing agents - oxalic acid, acidic hydrogen peroxide, iron (II) sulfate and hydroxylammonium sulfate - sodium chloride - before adopting the latter. In most cases, the final reduction of Hg (II) to Hg (0) is accomplished with $SnCl_2$, but in one case (MacPherson _et al._, 1982) alkaline $NaBH_4$ was used.

A trend toward simplification of the digestion is evident in more recent work.

Randlesome and Aston (1980) proposed a digestion procedure for Hg in sediments which uses HNO_3 + HCl (9 + 1). Tin (II) chloride is added to the sample directly after the digestion. Production of foam by samples rich in organic matter is avoided by adding liquid paraffin immediately after adding $SnCl_2$. Several other authors have recently reported using simplified digestion procedures for determining total mercury in sediments. For example, Langston (1982) refluxed sediments with concentrated nitric acid, claiming that greater than 95% of the total Hg is usually extracted. Bloom and Crecelius (1983) tested their two-stage gold amalgamation method for total mercury in seawater by the use of nitric acid digested biological and geological standards. They were able to achieve recoveries of mercury from marine sediment reference materials within the 95% confidence limits of the certified value.

There are a number of non-automated methods for measuring total mercury in sediments. Most of them appear to be minor modifications of the original method of Hatch and Ott (1968). The methods given in Plumb et al. (1981), EPS (1979), and Walton (1978) are all similar, being mainly abstracted from the works of Agemian and Chau (1975b) and Ure and Shand (1974). Agemian and Chau (1975b) developed a comprehensive digestion procedure and adapted it to the automated cold vapour technique of mercury analysis. The Ure and Shand (1974) method differed from that of Hatch and Ott (1968) in that the mercury in the sample solution was first partitioned between the liquid phase and a fixed volume of air by agitation before the air was passed through the measuring cell, as this was considered to be more reproducible than bubbling immediately after addition of the $SnCl_2$. Ure and Shand (1974) recommended a digestion technique similar to the one of Iskandar et al. (1972) which employs a nitric-sulfuric acid mixture for digestion followed by permanganate oxidation. The digestion of Agemian and Chau (1975b) is also similar to Iskandar et al. (1972) except that persulfate and permanganate are used together to ensure complete oxidation of organic matter. Both procedures employ hydroxylamine to reduce excess oxidant and MnO_2, which could otherwise sequester Hg (II) by coprecipitation. Accuracy of the method of Ure and Shand (1974) was verified by comparison with an oxygen flask combustion method. The analytical range for Ure and Shand's method is 10 - 500 ng/g, which is nearly the same for Agemian and Chau's method. Cranston and Buckley (1972), who also used manual CV - AAS (slightly modified from Hatch and Ott) reported a somewhat lower value (2 ng/g) for the minimum concentration that can be confidently determined.

The recently reported method of MacPherson et al. (1982) employs nitric + hydrochloric acid (1 + 1) to digest sediment samples and $K_2Cr_2O_7$ for oxidation to ensure that mercury remains in the 2 + oxidation state. Mercury (II) is reduced to elemental mercury with sodium borohydride in alkaline solution. This reductant is said to be more effective (less subject to interferences) than $SnCl_2$ in acidic solution. Through the use of specially purified reagents, the blank was minimized, and a detection limit of 4.7 ng/g was realized. The accuracy of the method was verified by analysing the standard reference sediments, MESS-1 and BCSS-1, by the proposed and by an alternative method.

The method of Agemian and Chau (1975b) is actually only semi-automated in that the digestion procedure must be performed manually; the resulting centrifugate is analysed automatically. The sampling rate is 20 samples per hour and the detection limit is 10 ng/g. Jirka and Carter (1978) have devised a method for Hg in sediments which could be described as being almost fully automated, and which has a detection limit of 100 ng/g. In this method a manual pre-digestion/wet oxidation with $HNO_3 + K_2Cr_2O_7$ is supplemented with an automated wet oxidation of the aqueous portion of the predigested sample with $H_2SO_4 + K_2S_2O_8$. It would appear that the wet oxidation step is able to attack only the finer grained material which would be held in suspension to a variable extent. The Jirka and Carter approach is an attempt to adapt the El Awady et al. (1976) method for Hg in water and wastewater to sediment samples.

2.3.3.1 Summary

Sensitivity is an important factor to consider in choosing a method for determining mercury in arctic marine sediments. From the concentrations of mercury determined in Beaufort Sea sediments (Table III-2.8), it would appear that the detection limits for the automated methods are usually marginal. Manual methods differ most importantly in sample digestion method. While a rigorous digestion of the kind used by Agemian and Chau (1975b, 1976) may not be necessary, it is nevertheless the standard with which others must be compared. The less rigorous procedures employed by Randlesome and Aston (1980), Langston (1982) and Bloom and Crecelius (1983) offer the advantage of a savings in time, but have not been well proven. The method of MacPherson et al. (1982) seems to be as good as any of the manual methods covered in this review. The digestion is relatively rapid and the instrumental determination has a sufficiently low detection limit. In addition, possible interferences were investigated and found to be unlikely to occur for real samples, and accuracy was verified for two standard reference marine sediments.

2.3.4 Methods for Arsenic

The global concentration range for arsenic in soils and sediments is 1 - 50 µg/g. Conventional flame AAS is not quite sensitive enough for determining arsenic at these concentrations. Although sensitivities are typically two to three orders of magnitude greater for the graphite furnace than for the flame, there have been relatively few reports of methods for determining arsenic in sediments or biological tissues based on the graphite furnace, partly because of the popularity of the hydride generation technique and partly because of problems inherent in the furnace determination (Brooks et al., 1981a). These relate to the volatility of arsenic, to the prevalence of matrix interferences, and to the reaction between arsenic acid and the carbon of the furnace. Brooks et al. (1981b) found that coating the graphite tube with tantalum carbide greatly improves the precision and accuracy of arsenic determinations. Nickel salts can be added to the sample as a stabilizer so that the charring temperature can be raised from 600 to 1400C; this is now a standard procedure. However, Chakraborti et al. (1980a) determined that nickel does not eliminate strong interferences due to the presence of Al, Na, K and sulfate. The method of standard additions may be able to compensate for matrix effects in sediment solutions. Caravajal et al. (1983) found that the use of nickel and the method of standard additions were essential for the attainment of maximum accuracy for arsenic determinations in sediment solutions.

Walsh et al. (1976) developed a method for determining arsenic in air particulate matter after low temperature ashing and dissolution in nitric acid. The addition of magnesium in combination with nickel was found to enhance sensitivity and control interferences, the most serious of which was found to be due to sodium sulfate. Analysis of various aerosol samples by the proposed method, using the method of standard additions and by neutron activation analysis were in reasonable agreement. Iverson et al. (1979, 1980) used the graphite furnace as a detector for column chromatography of sediment solutions. Arsenate, arsenite, MA and DMA were leached from river sediments using a soil phosphorous extraction procedure and separated on a column of AG50W-X8. It was found that the addition of $Ni(NO_3)_2$ to eluate solutions before flameless AAS analysis removed organic interferences and normalized the response among the various arsenic species. The sensitivity of the flameless AAS technique (a detection limit of 2 µg/L) would be adequate for determining arsenic at the lower end of the concentration range.

An alternate approach to the determination of arsenic with some potential advantages is by electrothermal atomization after solvent extraction. The methods of Chakraborti et al. (1980b), Puttemans and Massart (1982) and Subramanian and Meranger (1981) could probably be applied to sediments after a suitable leach or digestion procedure. The authors have shown that interferences due to matrix components present in drinking, river, mineral and sea waters are adequately removed by the solvent extraction methods. Arsenic can also be measured by generation of arsine with sodium borohydride, trapping of the gas in a small volume of chloroform/ephedrine solution of silver diethyldithiocarbamate, and injection of arsenic-containing solution into a graphite furnace. Using this technique, Shaikh and Tallman (1977) obtained a detection limit of <0.2 µg/L for a 50 ml sample of water. The method has not yet been applied to sediment digests however. The amount of arsenic absorbed by the silver DDC solution can also be determined spectrophotometrically at 535 nm (APHA-AWWA WPCF, 1980), but this is cumbersome, relatively insensitive and subject to interference from antimony.

Pierce and Brown (1977) have shown that interference effects are greater with graphite furnace than with hydride generation atomic absorption techniques. In comparison with graphite furnace methods, most hydride generation methods are 1-3 orders less sensitive on a signal per gram of analyte basis, but more sensitive on a signal per unit concentration basis. For these reasons, hydride-based analytical techniques are more popular for analysis of trace metal solutions of widely varying composition. Agemian and Bedek (1980) developed a semi-automated hydride generation method for determining total arsenic and selenium in sediments. The automated apparatus consists of a multi-channel peristaltic pump and a manifold for transferring, mixing and reacting the sample and reagent solutions. Arsine is generated continuously and transferred to an elecrothermal atomizer made from a T-jointed silica tube wrapped with resistance wire. The system is a modification of the one described by Agemian and Cheam (1978). The sediment samples are totally digested with nitric, perchloric and hydrofluoric acids in a Teflon beaker. Potassium permanganate and persulfate are also added to ensure that conditions are sufficiently oxidizing so that As and Se will not be lost by volatization. The automated hydrochloric acid prereduction step prevents interference from residual permanganate and persulfate during the generation of metal hydrides with sodium borohydride. Spiking experiments showed good recoveries of various organo-arsenic and organo-selenium compounds. Several standard soils and sediments were analysed for As and Se by the proposed method, and the results were deemed accurate. Precisions obtained for As and Se at 1.2 and 0.6 µg/L respectively were about 5%.

Vijan et al. (1976) developed a semi-automated method for total arsenic in soil and vegetation samples with a detection limit of 0.15 µg/g. The nitric-perchloric acid digestion procedure employed does not attack silicates. Inorganic spikes were completely recovered, and accurate results were obtained for NBS orchard leaves. Forty samples a day can be analysed. Rubeska and Hlavinkova (1979) reported using the MHS-1 automated hydride system (Perkin-Elmer) to determine total arsenic in rocks and soils. The samples were attacked with aqua regia. Accurate results were obtained for 10 standard rocks, but a low recovery was obtained for one sample due to incomplete dissolution of the arsenic present. Penrose et al. (1975) measured arsenic in contaminated sediments from Moreton's Harbour, Nfld. by a hydride generation/silver DDC spectrophotometric method. The sediments were digested with nitric and sulfuric acids and the arsenic preconcentrated by coprecipitation with ferric hydroxide. Langston (1980, 1983) measured arsenic (III) and (V) in water, suspended particulate matter and bottom sediments from several U.K estuaries using a hydride generation system which included a liquid nitrogen cold trap. Sediment samples were extracted with lM HCl or 25% acetic acid to provide a nominal value of the 'biologically available' fraction or refluxed for 2 hours with concentrated HNO_3 to provide a 'total' extract.

Maher (1981) developed an elaborate method for determining inorganic and methylated arsenic species in marine organisms and sediments. In outline, the sediment method consists of a two-stage selective extraction of arsenic from the sediment sample followed by a two-fold solvent extraction to isolate inorganic arsenic as As(III), MA and DMA. The arsenic species are then separated on a column of Dowex 50AG-X8. The arsenic is determined in each fraction by reduction to the corresponding arsine in a zinc reductor column and decomposition of the arsine in a graphite furnace of an atomic absorption spectrometer. In the extraction procedure for sediments, an initial leach with 1 M HCl is used to remove the bulk of carbonate and hydrated oxide phases before extraction with .1 M NaOH + 11 M NaCl solution. It was found that most of the inorganic arsenic was extracted by the hydrochloric acid solution, while significant amounts of the methylated forms were found in the sodium hydroxide extracts.

Several ICP-based methods have been reported for determining arsenic and other hydride-forming elements in soils and sediments (de Oliveiria et al., 1983; Goulden et al., 1981; Pahlavanpour et al., 1980). The methods use continuous hydride generation systems similar to those used with atomic absorption spectrometers, and the detection limits that can be achieved by either system are similar (Pruszkowska et al., 1983). The prinicpal advantage of the ICP system is the possibility of simultaneous multi-element determination. The techniques used by the authors for digesting their sample vary. Goulden et al. fused 0.2 g of dried sediments with 2 g of NaOH in a zirconium crucible, dissolved the melt in 40 mL .02 M HCl and precipitated silicic acid by adding 20 mL concentrated HCl and heating to 90 C for one hour. Pahlavanpour et al. leached .25 g samples with 5 mL portions of concentrated HCl in tightly capped (Sovirel) test tubes placed in an aluminum heating block held at 150 C for 2 hours. This kind of sample attack is intended to dissolve metals bound to precipitated iron (III) oxide minerals, and to provide a method suitable for high throughput work required in applied geochemistry. The method yielded slightly low results on standard rocks in some cases, however. About 200 samples can be run by one operator in a 2 day cycle. de Oliveira et al. tested four different sample dissolution techniques: 1) NaOH fusion, 2) KOH fusion, 3) $HNO_3 + HClO_4 + HF$ and 4) $HNO_3 + HClO_4 + H_2SO_4$. All four digestion procedures were found to be suitable for arsenic, but only KOH fusion yielded accurate results for antimony and only methods 1 and 3 were appropriate for determining selenium in marine sediments.

The method of hydride generation employed by Pahlavanpour et al. was developed previously by Thompson et al. (1978a, b). Organic matter interferes with the recovery of bismuth. Also, potassium iodide is required to reduce the copper interference with bismuth. The limit of detection is about 1 µg/L of As in solution or 0.1 µg/g as As in sediment. The hydride generation system of Goulden et al., is similar to the one described by Goulden and Brooksbank (1974), except that sodium borohydride is used instead of Al + HCl. Goulden et al., tested for interferences in the hydride step by spiking sediment solutions with As and Se; quantitative recoveries were obtained. Plasma stability was improved by using a modified torch and a temperature control system on the spectrometer enclosure. Detection limits were reported to be .08 µg/L for As and .12 µg/L for Se. A pre-reduction step was found to be necessary for selenium (de Oliveira et al., 1983). Considerable differences in sensitivities were found for the two oxidation states of As and Sb. The detection limits for As, Sb and Se are 1, 1 and 0.5 µg/L, respectively.

2.3.4.1 Summary

The principal methods for determining arsenic in sediments have been summarized in Table III-2.15. In comparing these methods, some generalizations can be made. The total attack methods yield complete recovery of arsenic, while the strong attack methods sometimes do not. In order to obtain an accurate value it is necessary to use either fusion or HF digestion. However a strong attack method will usually give a close approximation to the total arsenic present. The closed tube, HCl decomposition method of Pahlavanpour et al. (1980) has a high extracting efficiency and is simple and rapid. Several authors have cautioned that loss of arsenic can occur during the digestion due to the volatility of As (III) halides. To ensure that this does not occur, digestions can be made in closed vessels or else strong oxidizing agents can be added. The anion exchange method of Maher (1981) could be used to measure the species of arsenic released by a moderate or weak attack.

The hydride generation technique is preferred to the direct graphite furnace technique, because it is faster, more sensitive and less subject to interference. Several solvent extraction methods are available which could eliminate interference problems in graphite furnace analysis, but they have not yet been applied to sediments. The continuous (automated) hydride generation methods have the advantage of requiring less attention, leaving the operator free to perform the digestions simultaneously. The manual equipment is less expensive however, which is a significant advantage when only few samples are to be run. Manual methods are potentially much more sensitive, but the detection limits of the automated methods are certainly adequate for arctic sediments (Table III-2.8). The continuous hydride generation/ICP methods are advantageous for the determination of 2 or 3 elements, because they are done simultaneously.

TABLE III-2.15

METHODS FOR ARSENIC IN SEDIMENTS

	METHOD SUMMARY	COMMENTS	METHOD REFERENCE
a)	**GF-AAS Methods**		
	$HNO_3 + HCl$ extraction; direct GF determination. Ni $(NO_3)_2$ plus standard additions used.	As in sediment. Accurate value obtained for NBS river sediment. Direct determination of As by GF-AAS may be subject to reproducibility and matrix interference problems.	Caravajal *et al.* (1983)
b)	**HG-AAS Methods**		
	Total digestion with $HF + HNO_3 + HClO_4$ in Teflon beaker. Automated system used after the digestion.	As and Se in soils and sediments. Permanganate and persulfate were added to prevent volatization of As (III) halides. As was accurately determined in 6 soil and sediment standards. C.V. for As is 5% at 1 µg/g.	Agemian and Bedek (1980)
	$HNO_3 + HClO_4$ digestion. Automated system used after the digestion.	As in soils. LOD: 0.15 µg/g. Inorganic spikes were completely recovered and accurate value obtained for NBS orchard leaves. 80 samples/2 day cycle.	Vijan *et al.* (1976)
	Aqua regia digestion. MHS-1 mercury/hydride system used.	As in rocks and soils. Accurate values obtained for 10 standard rocks.	Rubeska and Hlavinkova (1979)
c)	**HG-ICP Methods**		
	NaOH fusion, HCl dissolution of melt. Automated system used after the digestion.	As and Se in sediments. LOD for As: 0.01 µg/g. Spiked recoveries verified and accurate values obtained for 6 reference soils and sediments.	Goulden *et al.* (1981)
	Three different total attack and one strong attack methods were tried. Automated system used after the digestion.	As, Se and Sb in sediments. All four digestions gave accurate results for As in NBS and NRC standard sediments. LOD for As: 0.1 µg/g.	de Olievera *et al.* (1983)
	Samples treated with conc. HCl for 2 hr. at 150°C in capped test tubes.	As, Sb and Bi in soils and sediments. High throughput method - 200 samples/2 day cycle. Accuracy tested on eight standard soils and rocks; method yielded low results in some instances. LOD for As: 0.1 µg/g.	Pahlavanpour *et al.* (1980)

2.4 Biota

2.4.1 Sample Preparation Methods

The subject of sampling, storage, and preparation of biological materials for trace element analysis has been reviewed by Sansoni and Iyengar (1980 a, b) and Katz and Jeniss (1983). Aspects of sampling and sample preservation which are of concern to the analyst are that the sample is representative of the material being studied and that its composition has not been altered by handling or storage.

Biological samples are usually preserved by freezing or drying; preservatives such as formalin are avoided. Samples are generally stored frozen without removing shells or hard outer coverings. Samples can be stored in plastic, glass or wrapped in aluminum foil although plastic (polyethylene) bags are the most commonly used container. Changes in composition can result from a change in sample weight due to evaporation (concentrations of toxic substances are sometimes given per unit dry weight), segregation of a heterogeneous mixture (freeze-thaw cycles should be avoided), diffusion of analytes between tissues within a given organism and chemical processes such as oxidation which might alter the distribution of metal species present in a biological sample. Samples of 5 -10 grams wet weight are often necessary for determining less abundant trace elements.

If tissues from individual organs are to be analysed, they should be removed from a living animal if possible, since necrochemical changes can be very rapid (J. Uthe, pers. commun.). If whole specimens are to be analysed, depuration may be necessary to avoid contamination from the contents of the digestive tract. This is usually accomplished by placing the organisms in clear seawater for a period of one or two days (J.A.J. Thompson, pers. commun.).

As all tissue samples will be heterogeneous, they should be homogenized before subsampling. Contamination arising from bottom sediments, dirt or dust, sampling tools, container surfaces, etc., must be avoided. Non-metallic tools and lab-ware made of polyethylene, polypropylene, Teflon, quartz, titanium, agate, boron nitride and corundum are commonly used. Any of these would appear to be suitable on the basis of the study by Heit and Klusek (1982). The effects of dissecting tools composed of various materials on the trace element content of marine bluefish and freshwater mussels was studied for As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sn, Te, V and Zn. No significant effects were found.

The preparation of solid biological samples generally involves fragmentation of the bulk material, grinding to the desired particle size and homogenization. Homogenization facilitates representative subsampling and rapid digestion of biological samples. Homogenization can be carried out on wet or dry samples. Carrondo et al. (1979) for example, homogenized wet sewage sludge using an Ultra-Turrax homogenizer which had a stator and rotor made of titanium. A freeze dried sample can be conveniently homogenized in an agate mortar. Generally, freeze drying has been reported to be satisfactory for most elements in a variety of different matrixes. However losses have been reported for mercury in fish and pons (brain) samples (Sansoni and Iyengar, 1980). Maher (1983) investigated trace element losses during lyophilization of selected macroalgae and marine flesh tissues. There were no significant losses of As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn. From 3 - 15% of chromium was lost from several specimens, however. Oven drying results in variable losses, up to 50%, for a number of elements (Sansoni and Iyengar, 1980a). Considerable losses of mercury from fish and algae during oven drying, even at temperatures as low as 60°C have been reported (Haraguchi and Fuwa, 1982). Specific procedures are given for preparing various kinds of aquatic plant and animal specimens for trace element analysis by FAO (1975, 1983) and EPS (1979).

It is usually necessary to carry out some kind of destruction or removal of the interfering matrix of organic matter from biological samples prior to the determination of trace elements by AAS. There are two main families of techniques:

1) those involving air or oxygen carried out at relatively high temperatures, and
2) those involving liquid reagents (acids) carried out at temperatures limited by the boiling points of those liquids (Gorsuch, 1976).

Low temperature ashing using activated oxygen is an alternate technique which has not yet gained widespread application. The problems encountered during the decomposition of organic matter are: 1) losses of the analytes due to volatization or fixation to solid components of the system and 2) contamination due to impurities contained in the reagents used.

Dry oxidation is carried out by ashing the sample in a crucible at high temperature, usually 450 - 550°C. The ash is then dissolved using a suitable acid. Large samples can be digested by this method and contamination due to reagents is reduced. The main disadvantage is that the high temperature and dry conditions increase the risk of losses by retention on the walls of the crucible and by volatization. Retention losses are caused by the formation of complex compounds between metal oxides and the material of the crucible. Volatization losses take place for inherently volatile metals like Hg or by the formation of volatile metal chlorides or by reduction to volatile metals, such as Cd. These losses can sometimes be reduced by adding suitable ashing aids such as sulfuric acid or a nitrate salt, which form non-volatile compounds with the metals in question.

Wet oxidation is generally preferred because it results in lower losses of trace elements as well as faster and often more complete removal of organic substances, but care should be taken to ensure that the reagents used are sufficiently pure. Contamination is usually related to the quantities of reagents used. Sulfuric acid, nitric acid, perchloric acid and hydrogen peroxide used singularly, sequentially or in combination are the most common liquid oxidizing reagents. Sulfuric acid is often used together with nitric or perchloric acid, which act as the primary oxidants. Sulfuric acid serves to make resistant materials more susceptible to attack by the primary oxidants, and increases the boiling temperature of the mixture. However, with systems containing sulfuric acid there is risk of loss of elements such as lead by coprecipitation of insoluble sulfates. The high boiling point of sulfuric acid also makes it difficult to remove the excess acid. Perchloric acid is an extremely effective oxidant and a mixture of perchloric and nitric acid has fewer disadvantages than other combinations of acids. This mixture has the property that the redox potential rises from a level where only the most labile materials are oxidized to the final fuming perchloric acid state. Perchloric acid has achieved a reputation for being a dangerous and unpredictable substance, but the properties which make it useful are also those which call for precautions in handling. Cantle (1982) has described the precautions required for the safe handling and use of perchloric acid, and he has also given a procedure utilizing nitric and perchloric acids for dissolving samples of dried (low-fat) biological materials. Materials containing fats and oils present special difficulties, as such substances can react violently with hot perchloric acid.

Wet oxidations are normally carried out by boiling in open systems (Kjeldahl flask), in which case volatile metals such as mercury and chromium (as CrO_2Cl_2) may be lost. To avoid this, the digestions can be carried out in Teflon bombs, by boiling with reflux or at lower temperatures. Feldman (1974) has given three procedures for wet-ashing various biological samples with perchloric acid in a 250 mL borosilicate volumetric flask supplemented with an asbestos-jacketed air condenser on a variable temperature hot plate. The essential features of the method are careful control of refluxing and evaporation by the use of temperature programming, the use of minimum amounts of reagents and the use of the same vessel for storage and digestion. Three types of behaviour were observed in wet ashing the samples, and three variants of the basic procedure were developed to accommodate them. The basic assumption of the method is that the dewlines of the volatile mercury or other metal species will be lower than those of the degradation by-products and hydrates of nitric and perchloric acid which are to be eliminated. The validity of the assumption was tested in two ways: by measuring the recovery of mercury compounds added to the sample before wet ashing and by comparing with mercury concentration values obtained by other methods. Virtually complete recovery of mercury was indicated by both tests.

A pretreatment technique which seems promising is to apply low temperature ashing to a freeze dried sample. A radio or microwave frequency discharge produces oxygen radicals which efficiently oxidize organic materials, typically at temperatures in the range of 100 - 150 C. The methods manual by EPS (1979) describes a procedure and gives the results of an experimental evaluation. There were little or no losses of the metallic elements measured (Na, Fe, Cu, Zn, Mn, Pb, Cd, Mg, Ca, Mo, Co and Sr). However, Hg, As and Se were not measured and the applicability of the method to these metals cannot be assumed. A disadvantage of low temperature ashing is that it is a relatively slow process, expecially for large samples.

Katz and Jeniss (1983) briefly reviewed about a dozen studies in which various wet and dry oxidation procedures for preparing tissues for analysis by AAS were compared. It is difficult to generalize from the results of these studies. While both techniques have been successfully applied, instances of contamination and loss are commonplace. It appears that each combination of specific element, type of sample, and method of decomposition is unique. It is essential therefore that laboratories whose data will be intercompared use the same procedures and that these procedures be thoroughly evaluated by interlaboratory comparisons.

2.4.2 Methods for Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

2.4.2.1 Flame AAS Methods

Pickford (1981b) reviewed the recent advances that have occurred in the trace inorganic analysis of foodstuffs. In recent years, AAS has been by far the most popular technique, with hydride generation and electrothermal AAS becoming increasingly popular. ICP - AES is also becoming a widely used technique for trace metals. In this section on benthic organisms, methods based on AAS and ICP will therefore receive the major emphasis. Because certain trace metals are often present in the acid digests of food materials at levels that are below the limits of quantification by flame AAS, methods which involve some form of preconcentration followed by flame AAS or methods which employ electrothermal atomization are often necessary. Ihnat (1982) has reviewed the applications of AAS to food analysis and provided procedures for the determination of individual elements (Na and K, Ca and Mg, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As and Se, Mo, Cd, Sn, Hg and Pb).

Evans et al. (1980) developed a method for the determination of Cu, Fe, Mn and Zn in foodstuffs in which organic matter is destroyed by wet oxidation, and the measurement is made directly upon the digest solution by flame AAS. The digestion is carried out in a Kjeldahl flask using nitric and sulfuric acid resulting in a final sulfuric acid concentration of 3 - 5%. Interferences from the major inorganic substances present in foods were extensively investigated and found to be a function of burner design, burner height and fuel-to-air ratio. When these operating conditions were optimized, the interferences were eliminated for the most part. Detection limits were derived based on reproducibility for five analysts and an extended series of measurements. For 10 grams of sample digested to produce 100 ml of solution, the detection limits in µg/g are: 1.0 for Fe, 0.2 for Mn, 0.1 for Cu and 0.4 for Zn. Recoveries of spikes added to the samples before digestion ranged from 92 -105%. Excellent accuracy and precision were demonstrated for Fe, Cu and Zn by the analysis of five standard reference biological materials. Manganese values determined by the procedure were slightly higher than the certified ones.

Agemian et al. (1980) advanced a direct flame AAS method for Cu, Zn and Cr in fish which enables a large number of high-fat fish samples to be digested simultaneously. The digestions are carried out with $HNO_3 + H_2SO_4 + H_2O_2$ on 5 g samples in test tubes placed in an aluminum hot block. A charring step incorporated in the procedure breaks down fats to carbon black, which is cleared afterwards by addition of hydrogen peroxide. The final solutions are 10% in H_2SO_4, which results in a 10 - 25% suppression of sensitivity for the three metals measured. This is compensated by treating the standards the same way as samples. Interferences from inorganic substances present in samples were not investigated. The detection limits for Cr, Cu and Zn are all 0.2 µg/g. Recoveries varied from 90 - 110%; accuracy and precision were demonstrated for three reference materials. Arafat and Glooschenko

(1981) developed a method for measuring Al, Fe, Zn, Cr and Cu in plant tissue based on flame AAS. Samples were digested with $HNO_3 + H_2SO_4 + H_2O_2$ in 125 mL Erlenmeyer flasks. Interferences were not investigated, but aluminum contamination arising from the anti-bumping granules used in the digestion was discovered. Also, when a solution of digested plant material was allowed to stand undisturbed for several hours, a white precipitate, later identified as silica, developed. Detection limits for the elements determined were quoted as 0.1 µg/g. Recoveries and accuracy were determined by analysing three standard reference materials.

Cadmium, Pb and Ni, among other metals, often occur in marine biological samples at concentrations which are too low to be satisfactorily determined by flame AAS directly. Solvent extraction of the digested sample followed by determination of the metal chelates in the organic solvent improves sensitivity by a factor greater than that of the volume ratio of aqueous and organic phases. This enhancement effect may amount to a factor of two or more depending on the metal and the combustible organic solvent. Stein and McClellan (1980) investigated a large number of solvent extraction systems to determine the most efficient systems to use for Ni, Mn, Ag and Cd and to determine the extent to which the sensitivity for the metals can be enhanced using solvent extraction - flame AAS. The system efficiency is based upon the completeness of extraction, sensitivity to pH, solution uptake rate, combustibility of the solvent, and enhancement of the absorption signal. The systems exhibiting the greatest absorption and broadest pH range were used to prepare standard curves for each element to determine the extent to which the detection limit could be lowered. For volume concentration factors of 100, the lowest aqueous phase concentrations (µg/L) which gave absorbance readings equal to twice the standard deviation of the noise levels were 1.0 for Ni, and 0.1 for Mn, Ag and Cd. The extraction methods were applied to determining Ni and Cd in environmental samples, including fish. Recoveries in the range of 95 -102% for standard additions were reported. No interferences, analyte loss or contamination problems were encountered.

Evans _et al_. (1978) and Agemian _et al_. (1980) applied the chelation/extraction technique to determine Cd, Pb and Ni in food and fish samples, respectively. The two methods are similar in detail and in overall performance. A mixture of two dithiocarbamate complexing agents is added to an unbuffered aliquot of digested sample solution and the metals extracted into a smaller volume of ketone. The concentrations of metal chelates in ketone solution determined by flame AAS against standards similarly extracted. The approximate detection limits (µg/g) for the two methods are: .02 for Cd, .05 for Pb and .05 for Ni. The method of Evans et al. is much more thoroughly validated and documented than that of Agemian et al. Evans et al. conducted a study of interferences from a comprehensive list of inorganic ions likely to be encountered in the analysis of foodstuffs. The results indicated that Fe^{3+} and Sn^{4+} at levels sometimes encountered caused a significant suppression of either Cd or Pb, and that Ni suffers similarly from Cu^{2+} and Sn^{4+} interference. Attempts to remove these interferences with masking agents were unsuccessful. Precision and accuracy were assessed with respect to the analysis of NBS standard reference materials and the recovery of spikes added to foods containing negligible amounts of the three elements.

The detection limits of the flame AAS methods reviewed are given in Table III-2.16. The values are generally consistent for the three methods. Comparing these detection limits with the ranges of metal concentrations for marine biological samples (Table III-2.17), it is clear that the methods may be sensitive enough to quantify the metals in some of the samples, but they are not sensitive enough for determining the lowest naturally occuring concentrations, with the possible exceptions of Fe, Mn and Zn. For these samples it may be necessary to resort to the graphite furnace.

2.4.2.2 GF-AAS Methods

Several possibilities exist for the determination of trace metals in biological materials by electrothermal atomization - AAS. The simplest and most rapid way is to directly inject a suspension of a homogenized sample into a graphite furnace. This technique has been extensively applied to physiological fluids. The sensitivity of electrothermal atomization - AAS is sufficient for the metals of interest in most samples but this technique is also prone to severe background and matrix effects. Carrondo et al. (1979) provided a method for Cd, Cr, Cu, Ni, Pb and Zn in sewage sludge in which the sample is diluted with 1% nitric acid and blended with an Ultra Turrax homogenizer. The sample absorbance is directly compared with aqueous standards. In a statistically designed experiment, the proposed method was compared with three other methods in which the samples were decomposed by wet or dry oxidation procedures. The proposed method's recoveries and precisions compared well with the other methods. Carrondo et al. however, recommended that the method should be initially evaluated at each wastewater laboratory and that the method of standard additions be used to check the results at intervals.

TABLE III-2.16

DETECTION LIMITS (μg/g) of FLAME AAS METHODS FOR BIOLOGICAL MATERIALS

	Agemian *et al.* (1980)[1]	Evans *et al.* (1978,1980)[2]	Arafat & Glooschenko (1981)[3]
Al	-	1.0 NE	0.1
Fe	-	0.2 NE	0.1
Mn	-	0.2 NE	-
Cu	0.2 NE	0.1 NE	0.1
Zn	0.2 NE	0.4 NE	0.1
Cr	0.2 NE	-	0.1
Ni	0.05 E	0.05 E	-
Cd	0.02 E	0.02 E	-
Pb	0.1 E	0.05 E	-

1 - μg/g, wet tissue basis, 100x dilution factor; highest value derived from three methods of calculating detection limits
2 - μg/g, wet tissue basis, method for calculating "practical limit of detection" described in the paper.
3 - μg/g, dry tissue basis, 100x dilution factor; "adopted detectable level", not defined in the paper.
NE - Not Extracted, directly analysed
E - Solvent Extracted
- - Not determined

TABLE III-2.17

TRACE METAL CONCENTRATIONS IN MARINE BIOTA
(µg/g dry weight)

Metals	Fish and Marine Products[1]	Marine Mollusks[2]	Marine Fish[2]	Marine Biological Std. and Ref. Materials[3]
Cd	0.01 - 5	0.1 - 300	0.002 - 5.	0.06 - 3.5
Cu	0.1 - 140	0.2 - 6500	0.15 - 6.6	4 - 63
Ni	0.02 - 1.7	0.2 - 50	0.01 - 7.2	1 - 1.9
Zn	2 - 1600	4 - 7000	2 - 224	33 - 852
V	0.05 - 5			0.05 - 5
Cr	0.01 - 0.4	0.2 - 300	0.1 - 29	0.6 - 1.3
Pb	0.03 - 1.7	0.1 - 350	0.1 - 29	0.5 - 2.1
Hg	0.002 - 2	0.03 - 13	0.01 - 16	0.05 - 0.5
As	0.1 - 60	0.3 - 15	0.1 - 290	2.6 - 13.4

1 - Ihnat (1982)

2 - Forstner and Wittman (1979), Appendix B

3 - Russell (1984), Table 7

A wet or a dry oxidative digestion would normally be expected to improve precision and reduce interferences. Depressed sensitivity and/or variable matrix interferences can still occur when digested biological samples are atomized in a conventional graphite tube, however. For example, Borg et al. (1981) found that the sample matrix resulting from $HNO_3 + H_2O_2$ digestion of fish liver tissue caused a depression of the atomic absorption signal for Cu and Pb, but not for Cd and Zn. The only way the former two elements could be accurately quantified was by the method of standard additions. The use of a L'vov platform and matrix modification can eliminate such problems for volatile elements like Cd and Pb. Fernandez et al. (1981) found that accurate results for NBS spinach, bovine liver and oyster tissue could be obtained using the L'vov platform with peak area integration. When the samples were atomized off the wall of the graphite tube, the signals were considerably suppressed or enhanced. Hinderberger et al. (1981) used the L'vov platform in conjunction with $NH_4H_2PO_4$ to determine Pb and Cd in blood, urine and six NBS reference materials. Matrix modification permitted charring at a higher temperature, and precision was markedly improved by the use of the platform. Without the platform - modifier system, response ratios for Pb in the NBS materials were .21 - .59 indicating severe interference: the ratios improved to .91 - 1.06 when the sample was atomized from the platform in the presence of $NH_4H_2PO_4$. The elimination of interferences and improved precision in the determination of Pb in biological materials through use of the L'vov platform with ammonium phosphate has been confirmed by Rains et al. (1982) and Koirtyohann et al. (1982).

The relative freedom from interference effects achieved for Pb and Cd through the use of the L'vov platform is realized to a lesser extent for the more refractory elements which have higher appearance temperatures. The performance of electrothermal atomization AAS can be dramatically improved for these elements through the use of tubes coated with pyrolytic carbon and lanthanum carbide. Lagas (1978) investigated the effect of pyrolytic carbon and lanthanum carbide coatings on the sensitivity and repeatability of the determination of Be, Ba and V in surface and tap waters. The tubes were treated with an argon - methane mixture to produce a pyrolytic coating of carbon and subsequently treated with lanthanum to produce a coating of carbide. For the recommended conditions, Be, Ba and V can be determined without memory effects and with detection limits of .01, 1, and 1 µg/L, respectively. Sensitivities and detection limits for Al, Co, Cr, Cu, Mo, and Ni were also improved by factors of 2 - 5, while those of Cd and Pb were unchanged. The lifetime of the coated tubes is also increased. This technique should be of considerable value when applied to the analysis of biological materials, especially for determining those elements (e.g. Be, Ba and V) which would otherwise be plagued with reproducibility problems.

A more time consuming method refinement based on GF - AAS is to separate and preconcentrate the metal of interest from the solution resulting from digesting a biological sample by solvent extraction, ion exchange or coprecipitation. Problems can arise from the difficulty of performing a solvent extraction from a solution which contains appreciable amounts of acid and/or residual oxidizing agents. Several examples of solvent extraction/GF - AAS methods are contained in the review of Ihnat (1982). Okuno et al. (1978) developed a method for Pb, Cd and Cr in which fish tissue is dry ashed and the metals extracted with APDC into MIBK and then back-partitioned into aqueous acid solution for analysis by GF -AAS. To ensure complete extraction of chromium, it was necessary to pre-oxidize the sample solution with $KMnO_4$ and to reduce the excess with NaN_3. Recoveries of Pb, Cd and Cr from fortified samples averaged 91%, 100% and 100%, respectively. Cary and Rutzke (1983) used ferric hydroxide to coprecipitate chromium from the solution resulting from digestion of plant tissue with $HNO_3 + HClO_4 + H_2SO_4$. The iron is then extracted into MIBK from an 80% dilute hydrochloric acid solution, and chromium is determined by GF - AAS. Recovery studies and the analysis of five NBS biological standards showed that the method is reliable. Loss of chromium did not occur in the wet oxidation step because the conditions necessary for the formation and volatization of CrO_2Cl_2 were not obtained. The method of Cary and Rutzke is modified from that of Chao and Pickett (1980) which uses a similar digestion and chromium is extracted with methyltricaprylylammonium ion into MIBK. Values were obtained for chromium in two NBS biological materials were within the 95% confidence limits of the certified value.

2.4.2.3 ICP-AES Methods

The application of inductively coupled plasma atomic emission spectrometry (ICP - AES) to the analysis of trace elements in biological materials is subject to the same basic limitations as geological materials (Section 2.3.2.1-C). These limitations are chemical (arising from digestion of the material and/or preconcentration of analytes) and instrumental (interferences that arise in the final determination by ICP). The detection capability of ICP is usually in the microgram per litre range for most metals. Trace metal concentrations in biological materials frequently occur in the microgram per kilogram range. Mineralization of a tissue sample by conventional wet or dry oxidation procedures usually involves diluting the concentration of analytes. Consequently, it is frequently necessary to preconcentrate the analytes if they are to be properly quantified by ICP - AES.

Jones et al. (1982) applied the Chelex 100 technique of Kingston et al. (1978) in the determination of Cu, Zn, Cd, Pb, Ni, V, Mo, Co and Al in plant and animal tissues. Major elements (alkali and alkaline earth metals and phosphorus) as well as transition metals that are not well behaved on the resin (e.g. Fe and Mn) are measured directly on a fraction of the acid-digested sample. This paper focuses mainly on the chemical aspects, i.e. the digestion and the Chelex separation techniques. Evans and Dellar (1982) applied a solvent extraction procedure to isolate Cd, Cr, Pb and Ni from acid digested food samples. The solvent extraction procedure is similar to the one developed by Evans et al. (1978) for the determination of these metals in foods by AAS. In the ICP method, the metals are extracted into chloroform which is then evaporated, the metal chelates are oxidized with HNO_3 and dissolved in a fixed volume of hydrochloric acid. The major trace elements (Fe, Mn, Cu and Zn) are determined directly in the digested sample solution. This paper concentrates on the instrumental aspects of the determination, giving a more or less complete treatment of nebulization, compound formation and the various kinds of spectral interferences.

Several methods have been developed which do not employ preconcentration procedures in analysing biological samples by ICP. Dahlquist and Knoll (1978) investigated various sample preparation procedures and concluded that the nitric-perchloric acid digestion of Feldman (1974) is best for multi-element determinations. The low solubility of alkali metal and ammonium perchlorates restricted the concentration of solutions introduced into the plasma to a practical limit of no more than 1 g of dry sample per 50 ml of solution. In general application of their method to biological samples, Dahlquist and Knoll found the normal levels of Cd, Pb, Co, Cr, Ni and V close to or below the limit of quantitative determination.

Various approaches to sample dissolution have been tried for application of ICP to biological materials. McQuaker et al. (1979 a, b) used $HClO_4 + HNO_3$ (with and without) HF while Jones et al. (1982) used $HNO_3 + HClO_4 + H_2SO_4$. In both cases the digestions were carried out in open vessels. Brzezinska et al. (1984) recommended closed vessel digestion, citing the advantages of: minimized contamination, faster decomposition and minimized volume of acids. The final concentrations of acid in the digested sample solutions of McQuaker, Jones and

Brzezinska are 3.5% 20% and 6%, respectively. The unusually high acid concentration was defended by Jones et al. on the grounds that minor differences in residual acid concentration between sample digests would be "swamped" by the perchloric acid used to dilute the sample, and would thus not contribute to variability in rate of sample transport to the plasma. Brzezinska et al. provided two digestion procedures for biological tissues. When the levels of elements in the sample are high enough, the rapid procedure, which dilutes the sample by 200 times (mL final solution/g of sample), can be used. When the levels are low a slower procedure, which incorporates an evaporation step and results in a sample dilution factor of 25 - 50, is required. Animal tissues are digested in HNO_3+ $HClO_4$; decomposition of plant material requires more $HClO_4$ and some HF when SiO_2 is present. Jones et al. found that about half of the NBS biological reference materials were totally solubilized by their procedure, while the rest which contain siliceous materials, are only partially solubilized.

The detection limits for the methods of Jones et al. (1982) and of Evans and Dellar (1982) are given in Table III-2.18. These data can also be compared with the detection limits of the McQuaker et al. (1979a) method for biological samples which does not involve a separation/preconcentration procedure (Table III-2.9). Comparing these three sets of detection limits, it seems that for most metals when there has been a preconcentration and separation of analyte from the acid digested sample matrix, about an order of magnitude improvement in detection limit results. Comparing the data in Tables III-2.16 and III-2.18, detection limits for the ICP methods are generally lower than those for the flame AAS methods. For some metals the ICP detection limits are almost an order of magnitude lower, but generally the detection limits are not very different for the two classes of methods. Comparing the ICP detection limits with trace metal abundances in marine biota given in Table III-2.17, it can be concluded that direct ICP analysis (without preconcentration) is not sufficiently sensitive to determine any of the elements of interest, except possibly Fe, Mn or Zn, at the lowest levels encountered in marine biological materials. Even with the order of magnitude or so improvement in detection limit resulting from preconcentration/separation, there will still be problems with quantification for some of the elements at their lowest levels. However for the majority of marine biological samples, the ICP detection limits will probably allow accurate quantification. Jones et al., who evaluated their method in application to 10 NBS biological reference materials, noted that accuracy for the determinations of Pb and Cd was seriously degraded near detection limits with some of the SRMs. Vanadium

TABLE III-2.18

DETECTION LIMITS (μg/g) OF ICP METHODS FOR BIOLOGICAL MATERIALS[a]

	Evans and Dellar (1982)[b]	Jones *et al*. (1982)[b]
Cu	0.08 NE	0.03 C
Fe	0.1 NE	1.0 NC
Mn	0.05 NE	0.5 NC
Zn	0.27 NE	0.03 C
Cd	0.04 E	0.03 C
Cr	0.06 E	-
Pb	1.2 E	0.3 C
Ni	0.18 E	0.03 C
Mo	-	0.03 C
V	-	0.18 C
Co	-	0.06 C
Al	-	0.4 C

NE - Not Extracted
E - Extracted
NC - Not Chelexed
C - Chelexed
- - Not determined

a) biological materials include NBS biological reference materials and foodstuffs

b) detection limits defined by the authors concentrations were below the detection limit for several of the NBS samples. Evans et al. concluded that the detection limits for Pb and Cd do not permit the determination of levels normally found in dietary foodstuffs.

2.4.2.4 Summary and Conclusions

There are few proven analytical methods for trace elements in marine biological samples. No comprehensive multi-element schemes are available for marine biota such as exist for marine sediments, although there is clearly a need for them. Methods for several trace metals in foods exist however, and there is no reason to assume that they would not serve well for marine biota or could not be expanded to include other elements. The main methods are compared in Table III-2.19. Some general conclusions can be drawn from the successes and failures of these methods.

Flame AAS and ICP methods are probably the best choice for routine analysis when concentrations are high enough and samples are large enough. ICP methods have an obvious advantage over AAS methods when a large number of elements are to be determined. After correction for spectral interferences, ICP is probably freer from interelement interferences than flame AAS. However, the ICP methods are not as common. The digestion used by Agemian et al. (1980) is preferred for animal tissues because it processes the samples simultaneously and can completely oxidize high fat content samples. For plant samples, the methods given by Brzezinska et al. (1984) are preferred because HF is included to prevent coprecipitation of certain analytes with silica.

When the concentrations are very low, as low as the minimum concentrations indicated in Table III-2.17, or when the sample size is limited to less than a gram, GF-AAS methods are required. The method of Borg et al. (1981) would probably be a good choice for most applications. The digestion is effective, samples are processed simultaneously without requiring much attention and sulfuric acid is avoided, as it should be for GF-AAS. The method of standard additions should compensate for matrix effects in most cases, but this must be shown. The L'Vov platform may eliminate the need for standard additions for volatile elements, and pyrolytic or lanthanum carbide coated tubes may improve performance substantially for non-volatile elements. It may be necessary to optimize GF conditions for new elements or different types of samples. In most cases, solvent extraction will not be necessary to achieve low enough detection limits or to reduce interferences sufficiently.

TABLE III-2.19

COMPARISON OF MULTIELEMENT METHODS FOR BIOTA

SUMMARY OF METHOD	ADVANTAGES	DISADVANTAGES	REFERENCE FOR METHOD
a) **Flame AAS Methods**			
Foodstuffs digested with $HNO_3 + H_2SO_4$. Fe, Mn, Cu and Zn determined directly. Ni, Cd and Pb determined after solvent extraction.	- Flame methods are faster than GF methods and less prone to interferences. - Preconcentration increases sensitivity for Ni, Cd and Pb. - More thoroughly validated than the method of Agemian et al. (1980). - Accuracy and precision demonstrated by analysing 5 SRM's.	- Requires 10 g of sample. - Detection limits of the flame methods are generally marginal for marine biota.	Evans et al. (1980)
Fish tissue digested with $HNO_3 + H_2SO_4 + H_2O_2$. Cu, Cr and Zn determined after solvent extraction.	- High fat content samples are completely oxidized. - Samples are digested simultaneously in an aluminum heating block. - Accuracy and precision demonstrated by analyzing 3 SRM's.	- Requires 5 g of sample. - Interferences were not investigated. - Digestion involves successive additions, charring step.	Agemian et al. (1980)
Plant tissue digested with $HNO_3 + H_2SO_4 + H_2O_2$. Al, Fe, Zn, Cr and Cu determined directly.	- Accuracy verified by analysing 3 NBS reference biological materials.	- Other workers have found that HF is sometimes needed to digest plant materials containing silica and that silicate occlusion is a possible source of error.	Arafat and Glooschenko (1981)
b) **GF-AAS Methods**			
Sludge sample homogenized with HNO_3. Cr, Cu, Ni, Zn, Cd and Pb determined directly.	- GF-AAS is more sensitive than flame AAS or ICP-AES and requires minimum sample size. - Simplest and most rapid method. Digestion step obviated by taking full advantage of charring ability of GF.	- Prone to interferences; GF conditions are critical; method of standard additions necessary.	Carrondo et al. (1979)

TABLE III-2.19

COMPARISON OF MULTIELEMENT METHODS FOR BIOTA (Continued)

SUMMARY OF METHOD	ADVANTAGES	DISADVANTAGES	REFERENCE FOR METHOD
Fish liver tissue dry digested with HNO_3+H_2O_2. Cu, Pb, Cd and Zn determined directly.	- Requires only .1-.5 g of sample. - High fat content samples are completely oxidized. - Samples are digested simultaneously in an aluminum heating block. - Method could be extended to include many other non-volatile metals. - Accuracy verified by analyzing NBS Bovine liver.	- Standard additions required for Cu and Pb to compensate for matrix effects. - Digestion involves successive additions, takes about 1 day.	Borg et al. (1981)
Fish tissue dry ashed in a Vycor crucible. Pb, Cd and Cr determined after solvent extraction.	- Dry ashing method more rapid, requires less attention and uses less acid than wet ashing method of Borg et al. (1981). - APDC/MIBK extraction eliminates salt matrix interference.	- Dry ashing techniques are more prone to volatilization losses. - Recoveries of Pb, Cd and Cr from fortified samples were demonstrated, but accuracy was not tested using SRM's.	Okuno et al. (1978)
c) ICP-AES Methods			
Foodstuff digested in HNO_3+ H_2SO_4. Fe, Mn, Cu and Zn determined directly. Cd, Pb, Cr and Ni determined after solvent extraction.	- More rapid than AAS methods when a large number of elements are determined. - Interferences were thoroughly investigated.	- Detection limits marginal for some metals in marine biota. - General lack of agreement with established levels in SRM's was found.	Evans and Dellar (1982)
Plant or animal tissue digested with $HNO_3 + HClO_4 + H_2SO_4$. Fe and Mn determined directly. Cu, Zn, Ni, Cd, Pb, and V determined after Chelex pre-concentration.	- More rapid than AAS methods when a large number of elements are determined. - Requires smaller sample (1 g) than flame methods. - Method tested by application to 10 NBS biological reference materials.	- Use of perchloric acid not popular - Detection limits marginal for some metals in marine biota. - Silicate occlusion is a source of error for Fe, Al and Mn in several SRM's if HF is not used in the digestion; HF interferes with Chelex pre-concentration of Al, Fe, Mn, Cr and Pb.	Jones et al. (1982)

2.4.3 Methods for Mercury

The determination of mercury in benthic organisms parallels that for sediments. Concentration ranges are similar in the two kinds of samples. The same basic variations of CV - AAS techniques that have been applied to sediments have also been applied to organisms. Sample digestion procedures are also very similar. In one case (Agemian and Chau, 1976), a single digestion procedure was recommended for both sediments and fish tissues. Because of these similarities, much of the information given in the section on sediments is applicable to biota.

Of the automated methods, that of Agemian and Chau (1975b) is suited for the determination of mercury in biological samples as well as in sediments, with a detection limit of 0.1 ng/ml or 0.01 µg/g. The automated method of El Awady et al. (1976) may also be suitable for tissue samples after homogenization and predigestion. Automated methods for mercury typically sample at the rate of 20 per hour, and digestion procedures which can process large batches of samples simultaneously are usually employed with them. The semi-automated method of Velghe et al. (1978a) for mercury in fish analyses at the rate of one sample per minute and has a detection limit of 0.15 ng/ml. The apparatus is atypical in that it is not a continuously flowing system. An aliquot of the sample digest is manually injected into an aeration cell; the rest is automated. The absorption cell is an open-ended Pyrex glass tube provided with a side tube for inlet of Hg vapour.

The rigorous digestion procedure of Agemian and Chau (1975b), employing nitric and sulfuric acid followed by wet oxidation with permanganate and persulfate, is effective but it is complex and relatively time consuming. In the same vein, Agemian and Cheam (1978) developed a digestion procedure employing sulfuric acid and H_2O_2 followed by wet oxidation with permanganate and persulfate for determining mercury and arsenic in fish. Perhaps the safest digestion with respect to volatilization is that of Feldman (1974) which utilizes an insulated condenser to prevent escape of Hg vapour (see 2.4.1). The digestion procedure adopted by the EPS (1979) is very similar to that of Agemian and Cheam. In order to streamline the digestion of fish tissue for automated determination of mercury, Velghe et al. (1978a) developed a simple procedure employing only $KMnO_4$ and H_2SO_4 which requires about 2 minutes for complete destruction of the sample. Complete recovery of inorganic and methylmercury added to 3 kinds of fish was verified. Duve et al. (1981) have provided a procedure in which 40 samples of fish are digested simultaneously in Nessler tubes with $HNO_3 + H_2SO_4 + V_2O_5$ in less than an hour. Sullivan and Delfino

(1982) have provided a digestion procedure for fish samples which utilizes a Technicon BD-4 block digester. Kuldvere and Andreassen (1979) gave two digestion procedures for the determination of mercury in seaweed. The two procedures give comparable results, but they differ in sensitivity to iodine intereference.

The use of strong alkali as a pretreatment for mercury determination in fish tissues is a significant new development. In the method of Chapman and Dale (1978, 1982) the sample is first warmed with 1.5 M KOH until dissolution or homogenization is evident. Then $KMnO_4$ is added and the sample is allowed to stand for 30 minutes. This causes almost complete sample oxidation. Dissolution is completed with H_2SO_4, and excess oxidant is reduced with oxalic acid before $SnCl_2$ is added to reduce mercury. The method is claimed to cut by 50% the time that is required for a H_2SO_4 -$KMnO_4$ pretreatment. Due to the possibility of a sample matrix effect, the method of standard additions was recommended for calibration. Accuracy was verified by analysis of NBS orchard leaves and IAEA fish homogenate. In the method of Tong and Leow (1980) a mechanically homogenized sample is dissolved with a solution of NaOH and NaCl in a test tube held in a water bath at 70 C. After cooling, an aliquot of the sample solution is pipetted into the reaction chamber and mercury is reduced with a solution of $SnCl_2 + CdCl_2$. The method was validated by examining the recoveries of $HgCl_2$ and CH_3HgCl added to shark meat, by analysis of NBS orchard leaves and bovine liver, and by comparison of results obtained by the proposed method with those obtained after sample digestion with $H_2SO_4 + HNO_3 + H_2O_2$. These tests established that the $SnCl_2$ - $CdCl_2$ reagent in alkaline medium was effective in reduction of inorganic and methylmercury in undigested fish samples.

In these studies, mercury was determined by manual techniques. Chapman and Dale (1978) devised a simple apparatus which uses water displacement to transfer the equilibrated mercury vapour at a constant rate to the absorption cell and which is capable of detecting 0.004 ng/mL. Tong and Leow (1980) developed a stationary CV - AAS apparatus which allows analyses to be run at the rate of ten per hour. A detection limit of 0.02 ng/mL was obtained for the short (45 mm) absorption cell.

Based on the semi-automated apparatus for total mercury described earlier, Velghe et al. (1978b) developed a selective method for determining both inorganic and methylmercury in fish. The method is based on the discovery, attributed to Magos (1971), that alkylmercury can be reduced to atomic mercury by Sn (II) in an alkaline medium and in the presence of Cd (II). In the proposed method, a sample of pretreated fish is introduced through a side arm into a specially fabricated aeration cell. Water and concentrated sulfuric acid are added, and the cell is agitated. After

30 seconds, $SnCl_2$ + $CdCl_2$ is added and the cell is agitated for 20 seconds more while Hg (II) is reduced to atomic mercury. After blowing the mercury vapour through the absorption cell, NaOH is added to the cell, and the remaining methylmercury is reduced. Pretreatment for samples of fresh fish consists of homogenization in a Moulinex mincer followed by warming the homogenate in a closed vessel by immersion in a water bath at 80°C for 15 minutes. This was done to shorten the time required for dissolution by water + H_2SO_4 in the aeration cell, and was not necessary for preserved fish. Except for pretreatment and sample introduction, the procedure is automated, the time required for analysis being about 2 minutes. The proposed method was applied to 7 different kinds of fish and shrimp. The inorganic mercury concentration (less than 0.01 µg/g) was too small for accurate quantification; methyl mercury ranged from 75 - 98% of the total. Accuracy of the proposed method was evauated by comparison with the results for total mercury obtained by the previously described H_2SO_4 + $KMnO_4$ wet oxidation method (Velghe et al., 1978a); the two methods were in good agreement.

2.4.3.1 Summary

The essential features of the main methods are summarized in Table III-2.20. In general, the automated methods are faster and require less attention but have inferior detection limits. Automated apparatus is also more expensive. Of the manual methods, the most rapid is that of Velghe et al. (1978b) which has the additional advantage of being able to determine both inorganic and methyl mercury. However, the accuracy and selectivity of the method has not been well validated, and for this reason is not included with the other methods for methyl mercury. The alkali pretreatment method of Tong and Leow (1980) is almost as rapid as the acid pretreatment of Velghe et al. (1978b) and the accuracy of the former method is well established. It would appear that the more rigorous and time consuming alkaline permanganate oxidation method (Chapman and Dale, 1982), is not required. The stationary CV -AAS technique of Tong and Leow is relatively slow. Therefore, it would seem that the Tong and Leow pretreatment method would be more advantageously combined with the Chapman and Dale water displacement CV - AAS technique which is faster and just as sensitive.

TABLE III-2.20

COMPARISON OF METHODS FOR MERCURY IN BIOTA

METHOD REFERENCE	SAMPLE PRE-TREATMENT	INSTRUMENTAL TECHNIQUE	COMMENTS
Agemian and Chau (1975b, 1976)	Manual digestion with H_2SO_4 + HNO_3; wet oxidation with permanganate + persulfate; hydroxylamine pre-reduction. A batch of samples can be processed in 2 hours plus overnight.	Manifold-type automated system; Sampling rate 20 per hour. L.O.D. 0.01 µg/g	For sediments or fish.
Agemian and Cheam (1978)	Manual digestion with H_2SO_4 + H_2O_2; wet oxidation with permanganate and persulfate; hydroxylamine pre-reduction. A batch of samples can be processed in 2 hours plus overnight.	Same as above.	For As and Hg in fish.
El Awady et al. (1976), Jirka and Carter (1978)	Manual pre-digestion with HNO_3 + $K_2Cr_2O_7$, takes ca. 1 hour plus overnight.	Manifold type automated system incorporating wet oxidation and pre-reduction steps. L.O.D. 0.1 µg/g. Analysis rate ca 30 per hour.	Has been applied to sludge and sediment samples, may be suitable for biological samples. Accuracy evaluated by comparison with other methods, no significant bias found. Problems can arise from solid material settling in the system.
Velghe et al. (1978a)	Homogenized sample manually digested with H_2SO_4 + $KMnO_4$, takes ca. 2 min; pre-reduction with hydroxylamine.	Automated aeration cell; takes about 45 sec.	Recoveries of inorganic and methyl mercury added to fish verified.
Velghe et al. (1978b)	Sample pre-treatment consists of cooking minced sample for 15 min.	Automated aeration cell, modified from above. Sample digested with H_2SO_4 in the cell; inorganic Hg reduced with $SnCl_2$ + $CdCl_2$, methyl Hg subsequently reduced by adding NaOH. Analysis time ca. 2 min.	Selective reduction method for determining inorganic and methyl mercury in fish. Accuracy of total Hg determination verified by comparison with method of Velghe et al. (1978a).
Chapman and Dale (1978, 1982)	Sample pre-treatment consists of: warming sample with 1.5 M KOH, add $KMnO_4$ and let stand for ca. 4 hr., add H_2SO_4 and let stand for ca. 3 hr., reduce excess oxidant with oxalic acid. Requires much attention.	Manual, uses water displacement to transfer equilibrated Hg vapour from reduction cell to absorption cell. L.O.D. ca. 0.5 ng/g.	For fish. Accuracy was verified by analysis of NBS orchard leaves and IAEA fish homogenate. Calibration by standard additions recommended.
Tong and Leow (1980)	Dissolution by heating dispersed sample with 30% NaOH + 0.6% NaCl for ca. 30 min.	Manual, stationary CV-AAS apparatus. Pre-treated sample reduced with $SnCl_2$ + $CdCl_2$. L.O.D. ca. 2 ng/g. Analysis rate of ca. 10 per hr.	For fish. Accuracy verified by spike recovery experiments, analysis of NBS orchard leaves and bovine liver and analysis by independent method.

2.4.4 Methods for Methylmercury

The most widely practiced methods for measuring methylmercury in environmental samples are based on gas chromatography with electron capture detection. These methods were introduced in 1966-68 and later reviewed by Sumino (1975) and Westoo (1975). The fundamentals of the methods are as follows: 1) hydrochloric acid is added to the homogenized sample converting methylmercury, bound to organic substances, to methylmercuric chloride which is then extracted into benzene; 2) the benzene phase is separated and extracted with an aqueous solution of cysteine which combines with the methylmercury, purifying it; 3) after addition of hydrochloric acid to the cysteine solution, the methylmercury is re-extracted into fresh benzene; 4) an aliquot of the final extract is injected into the gas chromatograph. The election capture detector is very sensitive (absolute detection limit of 10^{-12} g Hg) and the resolving power of the gas chromatograph makes the method very selective for methylmercury. Sumino and Westoo discuss in considerable detail the extraction procedure and whether or not the final extract contains all the methylmercury without loss or degradation. The authors advocate two slightly different extraction procedures, and Westoo suggested that this may be responsible for the discrepancy in methylmercury results (for fish), obtained by Swedish and Japanese workers.

Krenkel and Burrows (1975) have critically evaluated the Sumino and Westoo methods and conducted experiments in order to develop a simple, concise method that could be adopted as a standard. The recommended analytical procedure contains the following modifications or improvements. Methylmercury is liberated from the sample by treatment with HBr and $CuSO_4$. The copper salt is added to assist the cleavage of methylmercury and the thiol groups of proteinaceous material. The extraction solvent used is benzene. Methylmercury is separated from benzene-soluble impurities by extraction into aqueous sodium thiosulfate. Treatment of this solution with a small volume of benzene produces the final extract, ready for analysis by GC. The detection limit for the method is 0.05 μg/g.

Hight and Capar (1983) reported the results of a collaborative study in which a method for the determination of methylmercury in fish by GC-EC was evaluated. In this method the homogenized sample is washed with acetone and benzene before HCl is added and the methylmercury is extracted into benzene. Organic interferences are removed by washing with acetone and benzene so that it is not necessary to purify the benzene extract; instead, it is concentrated on a Kuderna-

Danish evaporator prior to analysis by GC. The method has a limit of detection of 0.05 µg/g, and the reproducibility (C.V.) was 3-13% for samples of swordfish, tuna, oyster and shrimp at levels from 0.15 - 2.5 µg/g. The method was adopted by the Association of Official Analytical Chemists (A.O.A.C.).

Rice et al. (1982) presented methods for methylmercury in fish, water, urine and sediments based on the use of a gas chromatograph interfaced to an atmospheric pressure active nitrogen (APAN) after-glow detector. This unusual detection system monitors the emission of Hg atoms at 253.7 nm, so it is highly specific. The sample is first washed with acetone as before, and methylmercury is extracted with methylene chloride. The extract is concentrated by evaporation before injecting it into the gas chromatograph. The limit of detection using the APAN detector is 0.4 ng/g in fish or sediments. Czuba et al. (1981) developed an effective method for quantitative separation of biologically incorporated methylmercury from animal, fish or higher plant tissues. The use of naturally incorporated methylmercury in recovery studies is much better than spiking the sample with a pure compound. The methylmercury was labelled with ^{203}Hg so that an absolute determination of the amount present before and after destruction of the tissue sample was posssible. The method that evolved consisted of pretreatment with KOH, then with HCl. Methylmercury is then extracted with a benzene solution of dithizone. The benzene extract is purified by extracting it with KOH, and the organomercurial compounds are separated by thin layer chromatography. This method is probably more laborious and less suited to routine analysis, but it is of interest to note that the extraction of naturally incorporated methylmercury was quantitative.

An alternative to gas chromatography in the determination of methyl mercury is cold vapour-atomic absorption spectrometry which, though not as selective, has comparable sensitivity. The method of Biscogni and Lawrence (1974) essentially combines the Westoo extraction with the Hatch and Ott (1968) CV - AAS determination. The precision for analysis of real samples of fish is < 4.1%, and the mean recovery efficiency is > 90%. The limit of detection was not reported. The final determination of methylmercury can also be performed by graphite furnace using the method of Shum et al. (1979). This method relies on an acetone wash followed by an acid cupric bromide treatment and extraction into toluene. The toluene extract is stabilized with dithizone before injection into the graphite furnace, or alternatively the methylmercury can be repartitioned into an aqueous solution of $Na_2S_2O_3$ which is more appropriate for the automated injection technique. The limit of detection of the method is 0.08 µg/g Hg in fish. Because these methods will extract other organomercurials, such as phenylmercury, as well as methylmercury they are subject to interference from extraneous organomercurials. Methylmercury does appear to be the dominant organomercurial in many natural systems, however.

Collett et al. (1980) developed a very sensitive method for methylmercury in fish involving steam distillation using a Markham semi-micro distillation unit. The homogenized sample is first subjected to an alkali digestion for 1/2 hour; then HCl and $CuSO_4$ are added prior to distillation which takes about 4 minutes. The distillate containing methylmercury is wet ashed in a Kjeldahl flask with H_2SO_4 + $K_2S_2O_8$ for 35-40 minutes, and mercury is determined by CV - AAS. The limit of detection is 5 ng/g which is generally superior to the GC-EC methods. Recovery of methylmercury spikes added to two fish species ranged from 95-100%, and the method will not extract phenyl or diphenylmercury. Also, inorganic mercury does not interfere. Cappon and Smith (1980) cautioned that methylmercury breakdown in sodium hydroxide digests of biological material can result in significant losses over extended storage periods. They recommended that sample digests be analysed immediately after preparation. It would seem that steam distillation offers some advantages over the solvent extraction procedures, i.e. it is faster and easier to perform, and it may result in as pure a product.

2.4.4.1 Summary

Methylmercury is commonly determined in fish and sediments by methods based on gas chromatography with electron capture detection. The basic extraction and clean-up procedure, originated in 1968 by Sumino (1975) and Westoo (1975), has been superseded by the modified procedure of Burrows (1975). The acetone and benzene wash method used by Hight and Capar (1983) eliminates the need for a clean-up step, thereby streamlining the procedure at no expense to the detection limit. The APAN detector offers a dramatic improvement in sensitivity over electron capture, but it is a very new and presumably not widely available technique. Methods based on CV - AAS are a reasonable alternative to GC-EC, offering equal or better sensitivity but less selectivity. The steam distillation/ CV-AAS method of Collett et al. (1980) is perhaps the best CV - AAS method, since it has a lower detection limit than the GC/EC methods yet appears to be quite selective for methylmercury. It has the disadvantage of requiring about 1 hour per sample, which makes it slower than the GC-EC methods. Steam distillation coupled with GC-EC detection would seem to be an improvement, but has not yet been tried.

2.4.5 Methods for Arsenic

Many methods exist for the determination of arsenic and other hydride forming elements in biological materials by hydride generation atomic absorption spectrometry (HG - AAS). The method of Agemian and Cheam (1978) for arsenic in fish tissue and a later version for arsenic and selenium (Agemian and Thomson, 1980) are typical of the semi-automated types. Agemian and Cheam digested homogenized tissue samples with H_2O_2 + H_2SO_4 for 30 minutes at 60°C, later adding permanganate and persulfate to ensure complete oxidation of organo-arsenic compounds. Agemian and Thomson used a combination of nitric, perchloric and sulfuric acids with an aluminum hot-block to completely dissolve samples of resistant, high fat content fish tissue. Nitric acid is a strong interferrent in the hydride generation step, and must be removed from the solution by boiling it off. Due to the sensitivity of the sodium tetrahydroborate reduction to the concentration of HCl used to pre-reduce the higher oxidation states of arsenic and selenium, different conditions and hence different manifolds are needed for the determination of the two elements. The accuracy of the procedure was verified by analysing fish samples spiked with organometal species, and by analyzing NBS orchard leaves and bovine liver. Precisions are typically 5 -10% for As and Se at levels of .25 - .50 µg/kg in fish. The semi-automated methods of Vijan et al., for As in plants (1976) has been already described in Section 2.3.4. Higher results were obtained using this method than using a silver diethyldithocarbamate method. Arafat and Glooschenko (1981) developed a method for arsenic in plant tissue based on the automated apparatus of Goulden and Brooksbank (1974). The samples are digested with HNO_3 + H_2SO_4 + H_2O_2. The detection limit claimed for the method is 0.5 ng/g, and the coefficient of variation for 10 determinations of arsenic in Sphagnum fuscum at 0.55 µg/g was 4%. Accurate values were obtained for arsenic in NBS orchard leaves, tomato leaves and pine needles.

Two examples of batch type HG - AAS methods are provided by Evans et al. (1979) and Kuldvere (1980). The method of Evans et al. (1979) which determines arsenic, antimony and tin in foodstuffs, is well documented. A wet oxidation procedure is recommended for most samples, but when arsenic is to be determined in samples of marine origin, dry ashing with $Mg(NO_3)_2$ is recommended. The dry ashing technique gave generally higher arsenic values for a variety of food samples. For convenience, the sodium tetrahydroborate reduction is started with the elements present in their higher oxidation state. The possible direct interference of 25 cations and anions was studied; only As, Sb and Sn caused significant perturbations. The detection limits derived by Evans et al. (1979) for As, Sb and Sn are .01 - .02, 0.2 and 0.1 µg/g, respectively. Kuldvere used the MHS-1 mercury/hydride system (Perkin-Elmer) to determine arsenic in seaweed. Samples were wet ashed with HNO_3 + H_2SO_4 in digestion tubes placed in an aluminum hot block. Hydrogen peroxide was added to achieve complete dissolution and to keep arsenic in the pentavalent state. The method is sensitive to oxidation state, which is controlled, and no other interferences were encountered.

In contrast to the recommendations of Evans et al. (1979) regarding sample digestion, Uthe et al. (1974) found that a V_2O_5 + HNO_3 + H_2SO_4 attack on fish and shellfish tissue gave higher values for total arsenic than did a MgO + $Mg(NO_3)_2$ dry ashing procedure similar to the one used by Evans et al. (1979). Recoveries of spiked methylarsenic compounds were also higher by the wet ashing than by the dry ashing procedure. Langston (1980) and Penrose et al. (1975) used the MgO + $Mg(NO_3)_2$ dry ashing technique to prepare marine biological samples for arsenic determination. Freeman et al. (1976) reported a simple, direct GF - AAS method for determining total arsenic in biological samples without previous oxidation. The proposed technique gave accurate values for aqueous fish extract solutions containing natural organo-arsenic compounds relative to results obtained by the HG - AAS method of Uthe et al. (1974) or by direct injection of the oxidized samples into a graphite furnace. The method of Freeman et al. (1976) would be valuable for quickly checking the effluent from a chromatographic column.

Ihnat and Thompson (1980) reported the results of a collaborative study in which an HG - AAS method for determining As and Se in foods was evaluated. The authors concluded that the method suffers from systematic and random error and recommended that it not be adopted by the Association of Official Analytical Chemists (A.O.A.C.). Holak (1980) presented a method for Cd, Pb, Cu, Zn, As and Se in foods, which has been adopted as official first action by the A.O.A.C. The samples for As and Se are first digested by nitric acid in a Teflon Bomb and later dry ashed with $Mg(NO_3)_2$ in a muffle furnace. The residue is dissolved in 8M HCl and KI is added to reduce As (V) to As (III). Finally, As and Se are determined by HG - AAS. The results of a collaborative study of this method confirmed the recoveries and precision.

Solvent extraction appears to be a viable alternative to hydride generation for the determination of total arsenic in biological materials by AAS. For example, Thiex (1980) provided a method which specifies wet digestion followed by reduction of As (V) to As (III) and extraction by diethylammonium diethyldithiocarbamate in chloroform. Final quantification is by GF - AAS. The method was validated with respect to accuracy, recovery, repeatability, ruggedness and interferences. Thiex (1980) observed that for some animal tissues, the digestion procedure is critical. A thorough digestion using $HNO_3 + HClO_4 + H_2SO_4$ gives better results than one using just $HNO_3 + HClO_4$. The other solvent extraction methods already described in Section 2.2.5 could presumably also be adapted to the determination of arsenic in biological materials.

The arsenic speciation method of Maher (1981) was applied to tissue samples of Mercenaria mercenaria and of several species of macroalgae. A major part of the characterized arsenic in the algae occurred as dimethylarsenate (DMA) or compounds which were degraded to this form during the analysis. The method for biological samples is the same as that for sediments, described in Section 2.3.4, , except that arsenic species are initially extracted from the tissues by refluxing with 0.1M NaOH for 24 hours. The method of Aggett and Kadwani (1983) for determining arsenic species in interstitial waters and aqueous plants is simpler. The sample of lake weed is homogenized with de-ionized water and centifuged. The supernatant is filtered before speciation. The solid material is then refluxed with methanol and ethanol, the extracts evaporated and the residues re-dissolved in de-ionized water for speciation. Ten ml aliquots of the extracts are processed separately on a column of SRA-70 anion exchange material. The eluate was collected in 5 ml fractions and the arsenic determined by HG - AAS. Aggett and Kadwani found almost no DMA or methylarsenate (MA) in the three species of lakeweed examined. The very sensitive arsenic speciation method of Andreae (1977) has been applied to marine macrophytes (Andreae, 1978). The basic method has already been described in Section 2.2.5. The adaptation to seaweed requires that the biological sample be digested with 2M HCl for 10 minutes in a Waring blender and centrifuged. The centrifugate is analysed by the same procedure for seawater samples. Andreae (1978) found significant quantities of MA and DMA in several species of algae.

A hydride generation/ICP - AES method for the determination of As, Bi, Ge, Sb, Se and Sn in foods has been reported by Hahn et al. (1982). The samples were digested with a 3/2/1 mixture of nitric, perchloric and sulfuric acids with NH_4VO_3 added as an oxidative catalyst. A spectral interference caused by CO_2 was corrected by measuring the background emission at a nearby wavelength. The extremely low detection limits attained were made possible by condensing the evolved hydrides in a liquid argon cold trap. Accuracy and precision were verified by testing spike recoveries and by analysing four NBS standard reference materials. Continuous hydride generation/ ICP methods have been reported for As and Se in fish (Goulden et al., 1981) and for As, Sb and Se in fish (de Oliveira et al., 1983). Both of these methods rely on the popular $HNO_3 + HClO_4 + H_2SO_4$ digestion of Agemian and Thomson (1980).

2.4.5.1 Summary

Table III-2.21 summarizes the main methods for determining arsenic in biological samples. Many of the considerations that apply to a choice of method for sediments also apply to biota since the instrumental options are basically the same. The methods for biota aim for the total arsenic present in the sample, and their accuracies are usually assessed by analysing appropriate standard reference materials. The solvent extraction method of Thiex (1980) appears to be a viable alternative to the hydride generation methods. All these methods have sufficient sensitivity to determine arsenic quantitatively at levels typically found in marine biota (Table III-2.17).

A variety of sample dissolution methods have been successfully applied. It is controversial whether to use a wet or a dry ashing technique; both techniques have apparently been successful. The $HNO_3 + HClO_4 + H_2SO_4$ digestion performed in tubes in a heating block (Agemian and Thomson, 1980) is a popular and efficient method. To ensure that arsenic is not lost, it is sufficient to perform the digestion in a closed vessel or to use sufficiently oxidizing reagents that arsenic is held in the pentavalent state.

Although interelement interferences were rarely encountered in the hydride generation step, reaction kinetics is a possible interferent. This is because As (III) and As (V) react with sodium borohydride at different rates. Some methods include a pre-reduction step to ensure that all arsenic is in the trivalent state, while others for convenience start the borohydride reduction with arsenic in the pentavalent state. The advantage of having a pre-reduction step is that the determination is then more sensitive.

2.5 Literature Cited

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TABLE III-2.21

METHODS FOR ARSENIC IN BIOTA

	METHOD SUMMARY	COMMENTS	METHOD REFERENCE
a)	**Solvent Extraction/GF-AAS Methods**		
	$HNO_3 + HClO_4 + H_2SO_4$ digestion. Reduction of As(V) to As (III) and extraction with NaDDC/chloroform. Chloroform layer directly injected.	As in biological materials. Accuracy well validated. Method is not affected by minor variations in procedure but Fe and Cu can interfere. Sensitive to about 0.04 µg/g.	Thiex (1980)
b)	**Continuous HG-AAS Methods**		
	$H_2SO_4 + H_2O_2$ digestion.	As in fish. Permanganate and persulfate added to prevent volatization loss. Accurate value obtained for NBS orchard leaves. C.V. of 15% for As in fish at 0.1 µg/g.	Agemian and Cheam (1978)
	$HNO_3 + HClO_4 + H_2SO_4$ digestion in tubes using aluminum hot block.	As and Se in fish. Different manifolds are necessary for the two elements. Accurate value obtained for NBS orchard leaves and bovine liver. C.V.'s of 5-15% for As in four samples containing 0.26 - 0.44 µg/g.	Agemian and Thomson (1980)
	$HNO_3 + HClO_4$ digestion in tubes using aluminum hot block.	As in vegetation. Complete recovery of inorganic spikes and accurate values obtained for NBS orchard leaves. 80 samples/2 day cycle.	Vijan et al., (1976)
	$HNO_3 + H_2SO_4 + H_2O_2$ digestion.	As in plants. LOD: 0.05 ng/g. Accurate values for 3 NBS biological reference materials.	Arafat and Glooschenko (1981)
c)	**Manual HG-AAS Methods**		
	Dry ashing with $Mg(NO_3)_2$.	As, Sb and Sn in foods. LOD: 0.01 - 0.02 µg/g for As	Evans et al. (1979)
	Samples pre-digested with HNO_3 in Teflon bomb, later dry ashed with $Mg(NO_3)_2$.	As and Se in foods. Precisions and reproducibilities for As were about 15% in a collaborative study of foods.	Holak et al. (1980)
	$HNO_3 + H_2SO_4 + V_2O_5$ digestion. Zn + HCl employed for reduction to arsine.	As in fish and shellfish. The wet digestion method gave slightly higher values than a dry ashing procedure.	Uthe et al. (1974)
	$H_2SO_4 + HNO_3 + H_2O_2$ digestion in tubes using aluminum hot block. MHS-1 mercury/hydride system used.	As in seaweed. Spike recoveries verified but accuracy not assessed. No interferences found.	Kuldvere (1980)
d)	**HG-ICP Methods**		
	Digestion of Agemian and Thomson, automated HG system used.	As and Se in fish. Accuracy was checked by comparison of results on fish samples with results obtained by HG/AAS method; no differences were found. LOD for As: 1 ng/g.	Goulden et al. (1981)
	Digestion of Agemian and Thomson, automated HG system used.	As, Sb and Se in fish. LOD for As: 50 ng/g. Three NBS biological reference samples were analysed; the results agreed with certified values with the exception of As in oyster tissue.	de Olivera et al. (1983)
	$HNO_3 + HClO_4 + H_2SO_4 + NH_4VO_3$ digestion; liquid Ar cold trap/ICP determination; non-automated system.	As, Bi, Ge, Sb, Se and Sn in foods. LOD for As: 0.4 ng/g. Accuracies verified for 4 NBS reference biological samples.	Hahn et al. (1982)

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3. METHODS FOR THE ANALYSIS OF ORGANICS IN SEDIMENTS AND TISSUES

3.1 Introduction

This section reviews methods for the determination of selected organic compounds in marine sediments and benthic biota. The recently published literature has been surveyed to identify the analytical methods commonly used for these analyses. Both instrumental and chemical methods are included where appropriate and the merits of each are considered. Procedures for the following types of analyses have been reviewed:

1. Total organic carbon (TOC)
2. Chemical oxygen demand (COD)
3. Petroleum hydrocarbons
4. Oil and Grease
5. Phenols
6. Chlorophenols
7. Chlorinated hydrocarbons (Pesticides and PCBs)

Where possible, methods have been evaluated in terms of known concentrations of the above compounds or parameters in arctic environments.

3.1.1 Sources of Information

Standard methods manuals, technical reports and research papers published in the last five years, and personal communications have been used as sources of information in the preparation of this review. The methods manuals published by the Environmental Protection Service, the Inland Waters Directorate, the U.S. Environmental Protection Agency and the American Public Health Association were found to contain procedures for the analysis of the contaminants of interest in sediments or biota. The relevant recent literature was identified through a computer search of two data bases supplied by the CISTI (Canadian Institute for Scientific and Technical Information) system, namely Aquatic Sciences and Fisheries Abstracts and Chemical Abstracts, 1972-84.

3.1.2 Types of Analysis

Methods of analysis for organics fall into two main categories: specific compound analysis and procedurally - defined analysis. In the specific compound-type analyses, individual analytes are resolved, identified and quantified. Procedurally-defined methods are used to determine a suite of compounds rather than individual components. The range of compounds determined is defined by the analysis procedure employed and individual components are not resolved. The results are reported as a "total" value. For example, a value for oil and grease gives the total weight of components determined by the oil and grease analysis procedure. No other information (e.g. individual oil and grease compounds or levels of non-oil and grease compounds) is obtained.

Some results are reported as an equivalent of individual components. For example, hydrocarbons determined by fluorescence or ultraviolet spectroscopic measurements cannot distinquish the individual components in a mixture. The calibration solution may contain only a single compound such as chrysene and the level of hydrocarbons reported in terms of "chrysene equivalents".

3.1.3 Methods of Standardization

Standardization is the determination of the response function of the analysis procedure, or the net signal as a function of the analyte concentration (ACS Committee on Environmental Improvement, 1980; 1983). The net signal can be a mass value, a titrant volume or an instrumental response. Three methods of standardization are commonly employed in organic analysis: internal standardization, external standardization and standard addition.

Internal standarization, the most widely used of the three methods, involves the addition of a known amount of a reference material to the sample. For quantification, the signal of the analyte is compared against the signal from the internal standard. The internal standard material is chosen for its ability to simulate the behaviour of the analyte of interest in the analysis procedure, but it must be a compound not present naturally in the sample. Deuterated analogues of the analyte of interest are often used for this purpose. The earlier in the analysis procedure that the internal standard is added to the sample, the more accurate the results.

External standardization methods use a separate reference material. A solution containing a known amount of the reference material is analyzed in the same way as an unknown sample. For quantification, the signal of the analyte is compared against the signal from the external standard. This comparison can be carried out in a variety of ways. One method determines the response in triplicate of at least three different concentrations of external standard. As these concentrations should bracket the concentration of the analyte in the samples, this method is most useful for analysis of a small number of samples or for a series of samples in which the analyte concentration does not vary greatly. In situations where a non-linear response is suspected, the response of as many different concentrations of standard as possible should be measured. If the response is known to be linear, then one calibration point could be well defined by measuring many replicates at that point. The advantage of the external standard method is that the calibration material is usually the analyte being measured. The disadvantage is that the matrix of the external standard solution may not match the matrix of the sample solution and may result in an error if there is a different response for the analyte in the two types of solutions.

In the method of standard addition, known amounts of analyte are added to aliquots (three to five) of the sample. Each of the solutions is analyzed in exactly the same manner and the results are extrapolated to a zero signal level to determine the concentration of analyte in the unknown sample. Unmatched matrix problems are eliminated, but several solutions must be analyzed for each unknown sample of interest. The method of standard addition may also lead to erroneous values if the added analyte behaves differently than the same analyte incorporated naturally into the sample. (Typically added analytes show higher recoveries leading to erroneously low analysis results.) Standard additions are the most time consuming standardization method.

3.1.4 Evaluation Criteria

There are few reference standards or materials for organic contaminants in sediments or biota. Thus there is generally no means of evaluating or comparing the accuracy of various methods. In this report the evaluation of method performance has been based on interlaboratory comparisons, intercalibrations, stated performance characteristics (sensitivity, limit of detection, precision), sensitivity to interferences, recovery of standards, ease of operation, complexity and cost.

Specific reference materials and intercalibration studies are described for each of the analytes in the following sections.

3.1.5 Literature Cited

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ACS Committee on Environmental Improvement, 1983. Principles of environmental analyses. Anal. Chem. 55: 2210-2218.

3.2 Total Organic Carbon (TOC)

Total Organic Carbon (TOC) is a procedurally-defined quantity encompassing a range of organic compounds. In coastal sediments, these may include complex carbohydrates, sugars, proteins, peptides and amino acids, aromatic and aliphatic hydrocarbons, fatty acids, humic and fulvic acids, steroids, terpenoids and tetrapyrroles of terrigenous or autochthonous origin (Cranwell, 1976; Simoneit, 1978; Olaussen, 1980).

TOC methods do not measure volatile, low boiling (B.P. $< 100^{\circ}C$) organics such as biogenic gases (e.g. methane) in anoxic sediments (Weliky et al., 1983) as these are lost in various purging and heating steps. These compounds are, however, considered to be a minor part of most ocean sediments. Some TOC methods are effective at measuring refractory organic carbon which is not accessible to solvent extraction. Suzuki et al. (1983) found that up to 44% of TOC determined in Japanese river sediments was non-extractable.

The measurement of TOC in sediments is useful for a variety of chemical and geochemical reasons. These include:

1. Normalization of levels of specific pollutants or compounds. Natural trace metal and trace hydrocarbon levels vary directly with TOC levels (e.g., Forstner and Wittman, 1983; Nriagu and Coker, 1980; Liu, 1976; Riley et al., 1983; Bates et al., 1984).

2. Indication of general organic pollutant input (e.g., Serruya, 1971; Kemp, 1971, Suzuki et al., 1983).

3. A tracer of organic dumped material (e.g., Iseki et al., 1984).

4. Global atmospheric/oceanic CO_2 balance (e.g., Heath et al., 1977).

TOC concentrations in marine sediments are variable though generally in the range of 1-4%. In very coarse or sandy sediment, TOC levels may be less than 1% while values may exceed 10% in some basins with restricted flow. For the Beaufort Sea, an inventory of chemical data gave a range of 0.6% to 8.7% TOC (Thomas et al., 1982). Data from Strathcona Sound, a fjord on Northern Baffin Island indicated a maximum TOC level of 4% (Thomas et al., 1983a).

3.2.1 Sample Storage

Sediment samples for TOC can be stored in clean polyethylene, polypropylene or glass containers (Plumb, 1981). Cleaning procedures are not specified in most procedures. A detergent wash followed by a distilled water rinse is probably sufficient. Moist, refrigerated (Riley et al., 1983) or frozen (Plumb, 1981) storage is recommended since dry storage may result in oxidation or volatilization of organic C.

3.2.2 Pre-treatment

Frozen sediment samples should be thawed gradually (Plumb, 1981). They are then dried and may be sieved or ground (Table III-3.1). The drying procedure does not appear to be a critical step as volatile compounds are generally not considered (Weliky et al., 1983). Both wet oxidation and dry combustion procedures, however, specify grinding or powdering of the sample. Kolpack and Bell (1968) note that finely ground sediment is hygroscopic and recommend cooling after drying and storage in a dessicator.

The choice of sample size will depend on the method of analysis and detection chosen (Table III-3.6) and on carbon levels in the sediment. Sandy sediments contain less organic material than clay and silt sediments and will require larger samples (Riley et al., 1983; EPS, 1979).

3.2.3 Methods of Analysis

TOC analysis is a two-step procedure involving the conversion of carbon to CO_2 and measurement of evolved CO_2 or of oxidant consumed. A crucial step in each procedure is the partition between inorganic C (as CO_2, CO_3^{2-} and HCO_3^-) and organic C (as a variety of organic compounds in various states of oxidation). As shown in Table III-3.3, a variety of approaches to TOC measurement are used. The various terms and abbreviations for different carbon fractions are summarized in Table III-3.2.

Organic C can be converted to CO_2 by either wet oxidation (Section 3.2.3.1) or dry combustion (Section 3.2.3.2). Inorganic C is also converted to CO_2, by acidification ($CaCO_3 + 2H^+ \rightarrow CO_2 + H_2O + Ca^{2+}$) or by dry combustion. Total

TABLE III-3.1

SAMPLE PRE-TREATMENT FOR TOC ANALYSIS

REFERENCE	DRYING TEMP.	DRYING TIME	PARTICLE SIZE
Gaudette et al. (1974)	Air-dry	-	80 mesh sieve*
Suzuki et al. (1983)	1. 50°C 2. 105°C	48 hr. 5 hr.	Ground, 20 mesh
Mills and Quinn (1979)	110°C	4 hr.	Ground to powder
Froelich (1980)	110°C	4 hr.	Ground to powder
Gibbs (1977)	50°C (under vacuum)	16 hr.	(Dried after acid leach)
Plumb (1981)	70°C	-	Ground
Kolpack & Bell (1968)	105°C	24 hr	Ground
Plumb (1981)	Air-dry	-	Ground, 100 mesh Sieve

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* Air-dried sample, non-ferrous sieve used to reduced Fe interferences.

TABLE III-3.2

COMMONLY USED ABBREVIATIONS

Abbreviation	
C-CO_3	Carbonate Carbon (i.e. derived from $CaCO_3$, $MgCO_3$,etc.)
C_{INORG}	Inorganic Carbon
C_{ORG}	Organic Carbon
C_{SUM}	Sum of Organic and Inorganic Carbon
C_{TOTAL}	Total Carbon
DC	Dissolved Carbon (DOC + DIC)
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
PC	Particulate Carbon (POC + PIC)
PIC	Particulate Inorganic Carbon
POC	Particulate Organic Carbon
ROC	Residual Organic Carbon (Acid-insoluble)
SOC	Soluble Organic Carbon (Acid-soluble)
TC	Total Carbon (TOC + TIC or PC + DC)
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
VOC	Volatile Organic Carbon

TABLE III-3.3

EXAMPLES OF TOC ANALYSES

METHOD	AUTHOR	TIC	TOC	TC	
Wet Oxidation/ Titration	Gaudette et al. (1974)	-	X_1		TOC only
Acid Digestion/ Wet Oxidation/ Instrumental	Weliky et al. (1983)	X_1	X_1	X_2	TIC + TOC = C_{SUM} C_{SUM} vs C_{TOTAL}
	Mills and Quinn (1979)	$\uparrow_1$	X_1		TOC only
	Suzuki et al. (1983)	X_1	X_1		TIC + TOC = C_{SUM}
Acid Digestion/ Dry Combustion	Gibbs (1977), Plumb (1981)	$\uparrow_1$	X_1		TOC only
	Froelich (1980)	$\uparrow_1$	X_1	X_2	TC - TOC = TIC
Acid Digestion/ Total Carbon Dry Combustion	Kolpack & Bell (1968) Calvert (pers. comm.)	X_1	-	X_2	TC - TIC = TOC
Differential Combustion	Heath et al. (1977)	X_1	$\uparrow_1$	X_2	TC - TIC = TOC
	Plumb (1981)	-	X_1	X_2	TC - TOC = TIC

X	CO_2 determined
↑	C removed, not determined
-	C not affected, not determined
1,2	Sample number

carbon (TC) in a sample can be determined by dry combustion.

The CO_2 evolved in wet oxidation, acid digestion and dry combustion can be measured in several ways. These include:

1)	Gravimetric	- CO_2 is absorbed on an ascarite column. The weight gain is measured.
2)	Volumetric	- The volume of gas before and after absorption of CO_2 by a KOH solution is measured.
3)	Non-dispersive Infrared (NDIR)	- The difference in IR absorption between CO_2 and a non-absorbing gas is measured.
4)	Thermal Conductivity	- A gas chromatographic approach is used with a thermal conductivity detector or the thermal conductivity of a gas stream is compared before and after removal of CO_2.

3.2.3.1 Wet Oxidation

Wet oxidation or wet combustion procedures utilize chemical oxidizing agents to convert organic carbon to CO_2. Current methods employ potassium dichromate/sulfuric acid ($K_2Cr_2O_7/H_2SO_4$) or potassium persulfate ($K_2S_2O_8$), in a modified DOC procedure. TOC is determined either by a) titration of the excess oxidant or b) measuring the amount of CO_2 produced.

Wet oxidants do not oxidize 100% of the organic carbon (EPS, 1979; Copin-Montegut and Copin-Montegut, 1972 - 1973). The oxidation efficiency varies between 70% and 90% depending on the sediment (EPS, 1979). Wet oxidation is not specific for organic carbon; potential interferences include iron (II), manganese (II), sulfide and chloride ions. Manganese oxides in the sediments can produce a negative error (EPS, 1979). However, high levels of inorganic carbon (up to 50% carbonate) do not interfere with the oxidation of the organic material (EPS, 1979).

a) Wet Oxidation - Titration

The wet oxidation - titration method for TOC is a modified Walkley-Black procedure (1934). It involves an exothermic wet oxidation of organic C with $K_2Cr_2O_7/H_2SO_4$ and titration of the excess dichromate with ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4).6H_2O$; Gaudette et al., 1974, EPS, 1979, Head et al., 1981) TIC and TC are not determined.

The chief advantages of the method are simplicity and its widespread historical use. Capital costs are the lowest of the TOC procedures and forty-fifty analyses can be performed per day by unskilled personnel with minimal training.

A major disadvantage of the method is that it may be an inaccurate indicator of absolute organic carbon content of the sediment and should be considered as yielding a procedurally-defined result. The EPS recommends designation of results as "Organic Carbon, chromic acid oxidation values". Besides the sources of error discussed above (incomplete oxidation of organic carbon and non-specificity of the oxidation inaccuracies may arise from standardization difficulties due to sediment organic carbon being at a different overall oxidation state than the organic carbon used to standardize the titration (Niino et al., 1969; Copin-Montegut and Copin-Montegut, 1972-73). Potentially interferring chloride ions can be removed by precipitation with $AgSO_4$ during the reaction (EPS, 1979) although this may also tend to co-precipitate organic material (APHA, AWWA, WPCF, 1980). Gaudette et al. assume that Cl-oxidation is quantitative and calculated a correction factor from the salinity of bottom seawater and the sediment water content. The calculated error, however, was found to be very small (~0.15% TOC for a 0.2 g sample).

Several comparisons of sediment TOC values from wet oxidation-titration analyses and dry combustion analyses have been reported in the literature. Gaudette et al. (1974) found wet oxidation titration results for lacustrine and shallow marine sediments to be comparable to those of the dry combustion method (acid digestion, dry combustion in a LECO induction furnace). Niino et al. (1969) found the titration method to be reproducible for a given environment but noted that titration values were consistently higher than dry combustion values. They attributed this to a high overall oxidation state of sediment carbon due to the oxidizing environment from which their samples came. Copin-Montegut and Copin-Montegut (1972-1973) applied a higher temperature (boiling point) dichromate oxidation/titration method to Particulate Organic Carbon (POC) measurements in offshore seawater and compared a dry combustion/infrared detection method. They determined that titration results must be multiplied by 1.09 ± 0.03 to be comparable to dry combustion values. The infrared method was judged to have superior accuracy and sensitivity.

b) Wet Oxidation - Instrumental Methods

Instrumental methods for TOC directly measure the CO_2 produced by wet oxidation (a list of the cited instruments is given in Table III-3.5). Prior to the wet oxidation of organic C, carbonate carbon can be removed by acidification of the sample with a non-oxidizing acid treatment. Examples are given in Table III-3.4. Organic carbon is then oxidized to CO_2 by wet oxidation with $K_2Cr_2O_7/H_2SO_4$ (Weliky et al., 1983; Suzuki et al., 1983) or $K_2S_2O_8$ (Mills and Quinn, 1979,; MacKinnon, 1978).

TABLE III-3.4
ACID DIGESTION OF INORGANIC CARBON

REFERENCES	ACID TREATMENT	COMMENTS
Weliky et al., 1983	H_3PO_4	O_2 bubbling
Mills and Quinn, 1979	H_3PO_4	Sonication, N_2 purging
Suzuki et al., 1983	$H_2SO_4/FeSO_4$	$FeSO_4$ is added to prevent or reduce oxidation of organic C (Kolpack & Bell, 1968).

TABLE III-3.5
COMMERCIAL INSTRUMENTS USED FOR TOC IN SEDIMENTS

INSTRUMENT	PRINCIPLE OF OPERATION	REFERENCE
LECO WR-12 Carbon Analyzer (also LECO CHN/600)	Induction furnace combusts dried sample at 1200°C. CO_2 is collected on a molecular sieve, then released by heating to 320°C. Can be modified to collect CO_2 from wet oxidation reaction.	
	Detection:	
	1. Thermal Conductivity (GC separation). (Figure III-3.1)	Weliky et al. (1983)
	2. Gravimetric - Ascarite absorption bulbs.	Calvert (pers. comm.)
	3. Volumetric - KOH absorption vessel	Kolpack and Bell (1968)
LECO CR-12 Carbon Determination	Resistance furnace combusts dried sample in O_2 at 1350° C. Detection by infrared (NDIR)	
Shimadzu 10-B TOC Analyzer	CO_2 dissolved in NaOH trapping solution is injected directly onto Inorganic Carbon tube (packed with glass wool wetted with 85% H_3PO_4). CO_2 liberated at 150°C. NDIR detection (Figure III-3.2)	Suzuki et al., (1983)
Oceanography International 0524A TOC Analyzer	CO_2 from persulfate oxidation in sealed ampoules is liberated as ampoule is pierced. NDIR detector.	Mills and Quinn (1979) Froelich (1980)
Beckman 15-A IR	CO_2 from persulfate oxidation in sealed ampoules is liberated as ampoule is pierced. NDIR detector (Figure III-3.3)	Strickland and Parsons (1972)
Carlo Erba 1106 CHN/OS Analyzer	Dried sample combusted in Cr_2O_3 reactor at 1600°C, 80% O_2. Detection by thermal conductivity (GC separation)	Mills and Quinn (1979)
Perkin Elmer 240C CHN/OS Analyzer	Dried sample combusted in O_2 at 1000°C. Detection by Thermal Conductivity. Also determines TIC on separate sample using H_3PO_4 digestion.	Plumb, (1981)
Hewlett Packard 185B CHN Analyzer	Dried sample combusted in induction furnace. Detection by Thermal Conductivity (GC separation). No longer available.	Bates et al. (1984) Wangersky and Zika (1978)
Coleman 33 CH Analyzer	Dried sample combusted in induction furnace in O_2 stream. Detection gravimetric, by absorbtion of CO_2 on soda-lime-asbestos.	Strickland and Parsons (1972)

The liberated CO_2 is usually measured by modifying or adapting a commercial carbon or CHN analyzer. There are two basic approaches: 1) wet oxidation in a reaction vessel as part of a closed system directly connected to a CO_2 trap or detector and 2) wet oxidation in a sealed ampoule using a modified DOC procedure. The CO_2 can be determined by any of the detection methods described in Section 3.2.3 above. Determination of evolved CO_2 eliminates errors due to non-specificity of the oxidation and to standardization difficulties, but incomplete oxidation of the organic carbon may still be a problem.

"Reaction Vessel" Methods

Weliky et al. (1983) proposed an acidification/wet oxidation method for direct determination of both TIC and TOC on the same sample for their procedure (Figure III-3.1). Evolved CO_2 is measured directly by a thermal conductivity detector using a modified LECO carbon analyzer. The instrument can also be used for dry combustion. (Other instruments which could be modified are the Carlo Erba Model 1106 CHN-OS Analyzer, Hewlett-Packard 185-B and Beckman IR). In this procedure CO_2 produced in the wet oxidation vessel from the acidification of inorganic C or the oxidation of organic C is purged by a stream of O_2 and collected on a molecular sieve. Interfering gases (H_2O, SO_2 and Cl_2) are removed by a condenser and series of traps. The CO_2 is released by heating the molecular sieve to 320°C and is measured by thermal conductivity.

There are several advantages to this method. Both TIC and TOC are determined on the same sample, reducing the number of separate samples required for replicates. Acid-soluble organic C will be retained in the vessel. The use of the LECO carbon analyzer with a combustion temperature at >1200° C also allows the measurement of C_{TOTAL} when the furnace is used in its original application. The detection system, however, is common to both the wet oxidation and dry combustion systems. The measurement of C_{TOTAL} also acts as a check on the value of C_{SUM} = TIC + TOC.

The major disadvantages of this technique are the high capital costs of the instrumentation (as much as $30,000) and possible low values for TOC due to incomplete oxidation of the organic carbon.

An alternative to thermal conductivity detection is volumetric determination (Schmied and Steiner, 1957; Hartmann et al., 1971; Kolpack and Bell, 1968). The volume of gas produced, usually a mixture of CO_2 and O_2, is measured. The CO_2 is

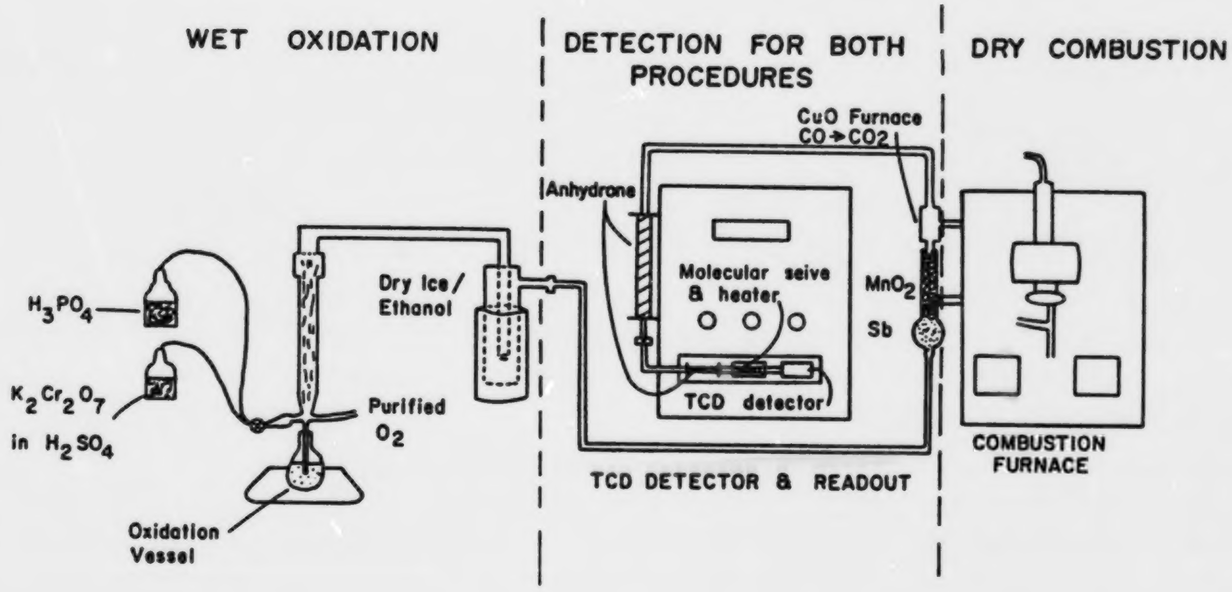
WET OXIDATION
DETECTION FOR BOTH PROCEDURES
DRY COMBUSTION
CuO Furnace
CO→CO2
Anhydrone
Molecular seive & heater
MnO2
Sb
Dry Ice/ Ethanol
H3PO4
K2Cr2O7 in H2SO4
Purified O2
Oxidation Vessel
TCD detector
TCD DETECTOR & READOUT
COMBUSTION FURNACE

FIGURE III-3.1 Modified LECO apparatus used for wet oxidation technique. Apparatus can be easily converted to wet oxidation from original LECO dry combustion technique. Thermal conductivity detection is used for both procedures (Weliky et al., 1983).

then absorbed in a KOH solution and the volume of the remaining O_2 is measured. The CO_2 evolved is calculated by difference. Again interferences must be eliminated. Kolpak and Bell (1968) remove water vapour with a condenser and H_2SO_4 scrubber, SO_2 with an MnO_2 trap and convert CO to CO_2 in a catalyst furnace. The cost of the furnace/gas buret system is lower than that of the thermal conductivity CHN analyzer (approximately $14,000).

Weliky et al. (1983) compared volumetric detection to thermal conductivity detection (as described above) in an intercalibration exercise. The results agreed to within 0.1% absolute TOC for carbon concentrations $< 0.50\%$ and within 4% relative for concentrations $> 1\%$ C. Volumetric determination is not as sensitive, however, and requires larger samples.

Another approach utilizes a Total Organic Carbon analyzer which has been designed for the determination of organic carbon in seawater. TOC analyzers are available commercially and may be more accessible than CHN analyzers. Suzuki et al. (1983) combine a wet oxidation method with analysis by a Shimadzu 10-B TOC analyzer normally used for water analysis. This instrument has both a dry combustion tube for Total Carbon (not used in this analysis) and an Inorganic Carbon tube. Aqueous samples are injected directly.

CO_2 produced by acidification of inorganic C or by wet oxidation of organic C is bubbled by a CO_2-free air stream into a NaOH solution (Figure III-3.2). Aliquots of this solution (with carbon as HCO_3^- and CO_3^{2-}) are removed by a syringe and injected into the inorganic carbon tube of the Shimadzu TOC analyzer. This tube is packed with glass wool wetted with 85% H_3PO_4. CO_2 is liberated by heating at 130°C and determined by a non-dispersive infrared detector. A relative standard deviation of 2-6% was obtained on sediment samples.

There are several advantages to this approach. Both TIC and TOC can be determined on a single sample, Cl^- and S^{2-} do not interfere with the analysis, the method can be adapted by laboratories with a direct injection TOC analyzer and, ampoule sealing is not required.

There are, however, certain disadvantages, namely: the optimization of amount of absorbing solution for TOC levels in the sediment and TOC analyzer sensitivity requires attention, the inorganic column tends to deteriorate due to injection of basic solution (maximum NaOH concentration is 0.2 N), a precise aliquot of the absorbant solution must be analyzed and, finally, Total Carbon is not determined as a check for C_{SUM}.

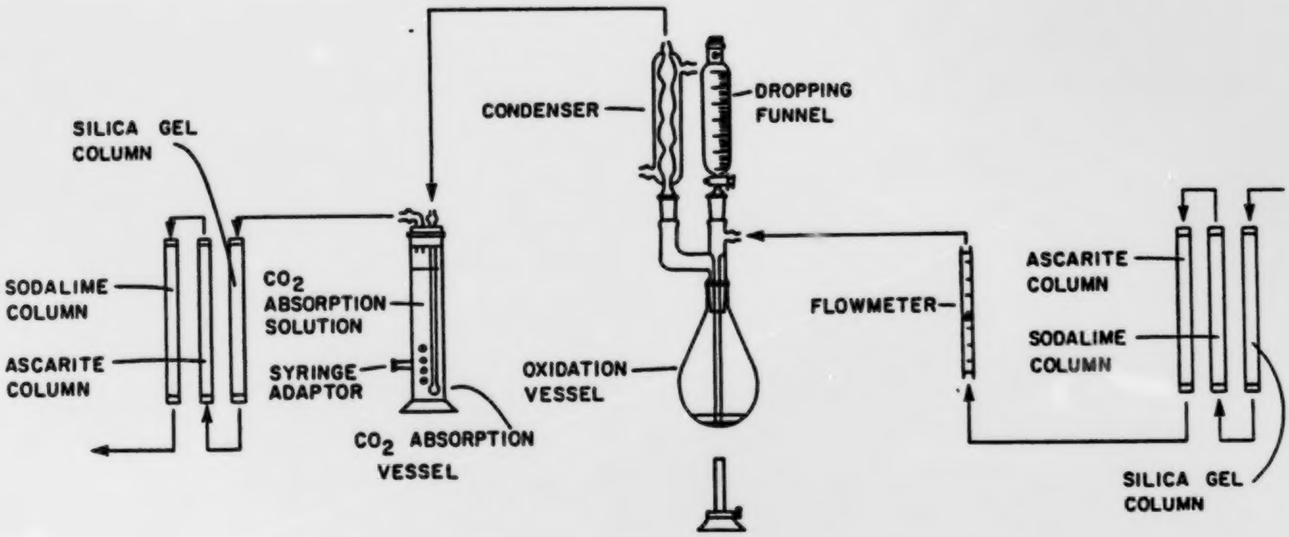
SILICA GEL COLUMN
SODALIME COLUMN
ASCARITE COLUMN
CO2 ABSORPTION SOLUTION
SYRINGE ADAPTOR
CO2 ABSORPTION VESSEL
CONDENSER
DROPPING FUNNEL
OXIDATION VESSEL
FLOWMETER
ASCARITE COLUMN
SODALIME COLUMN
SILICA GEL COLUMN

Figure III-3.2. Apparatus used for wet oxidation technique with absorption of evolved CO_2 in NaOH solution. Analysis of CO_2 in solution is by direct injection TOC analyzer (Suzuki et al., 1983).

Sealed Ampoule Method

This method offers an alternative to the relatively expensive CHN analyzers by adapting a TOC analyzer used for water analysis. Mills and Quinn (1979) adapted the modified Menzel and Vaccaro (1964) persulfate oxidation procedure for Dissolved Organic Carbon (DOC). As discussed previously, inorganic C is removed by acidification with H_3PO_4, sonication and N_2 purging after the addition of the oxidizing agent (premature oxidation of organic is negligible.) The samples are oxidized with $K_2S_2O_8$ in sealed ampoules at optimum conditions of 110°C for 3 hours. The CO_2 produced is measured with a non-dispersive infrared detector (NDIR) (Figure III-3.3). Five sediment samples containing between 0.25% and 6.35% TOC were analyzed (3 to 6 replicates each) and the average relative standard deviation was 11.4%. For comparison, the same samples (after acidification) were analyzed by a high temperature oxidation method, using the Carlo Erba 1106 CHN analyzer. The means of the TOC values for the five samples were the same by both methods at the 95% confidence level. However a lower relative standard deviation (5.1%) was achieved by the dry combustion method.

An advantage of the sealed ampoule method is that ampoule samples can be processed in batches and sealed ampoules can be stored indefinitely after the oxidation. The method can also be easily adapted by laboratories with DOC capabilities.

Problems with this approach can arise however, as a result of the skill required in sealing the ampoules. Also the method does not determine TIC or TC and incomplete oxidation of organic carbon may cause low TOC results.

3.2.3.2 Dry Combustion

Dry combustion methods are instrumental and involve oxidation of dried sediment samples at high temperatures, converting inorganic and/or organic carbon to CO_2. The partition of inorganic and organic carbon is again crucial. Three approaches have been used: a) Acid Digestion/Dry Combustion, b) Acid Digestion and Total Carbon Dry Combustion, and c) Differential Combustion.

a) Acid Digestion/Dry Combustion

An acid treatment step is employed to convert inorganic C to CO_2. The CO_2

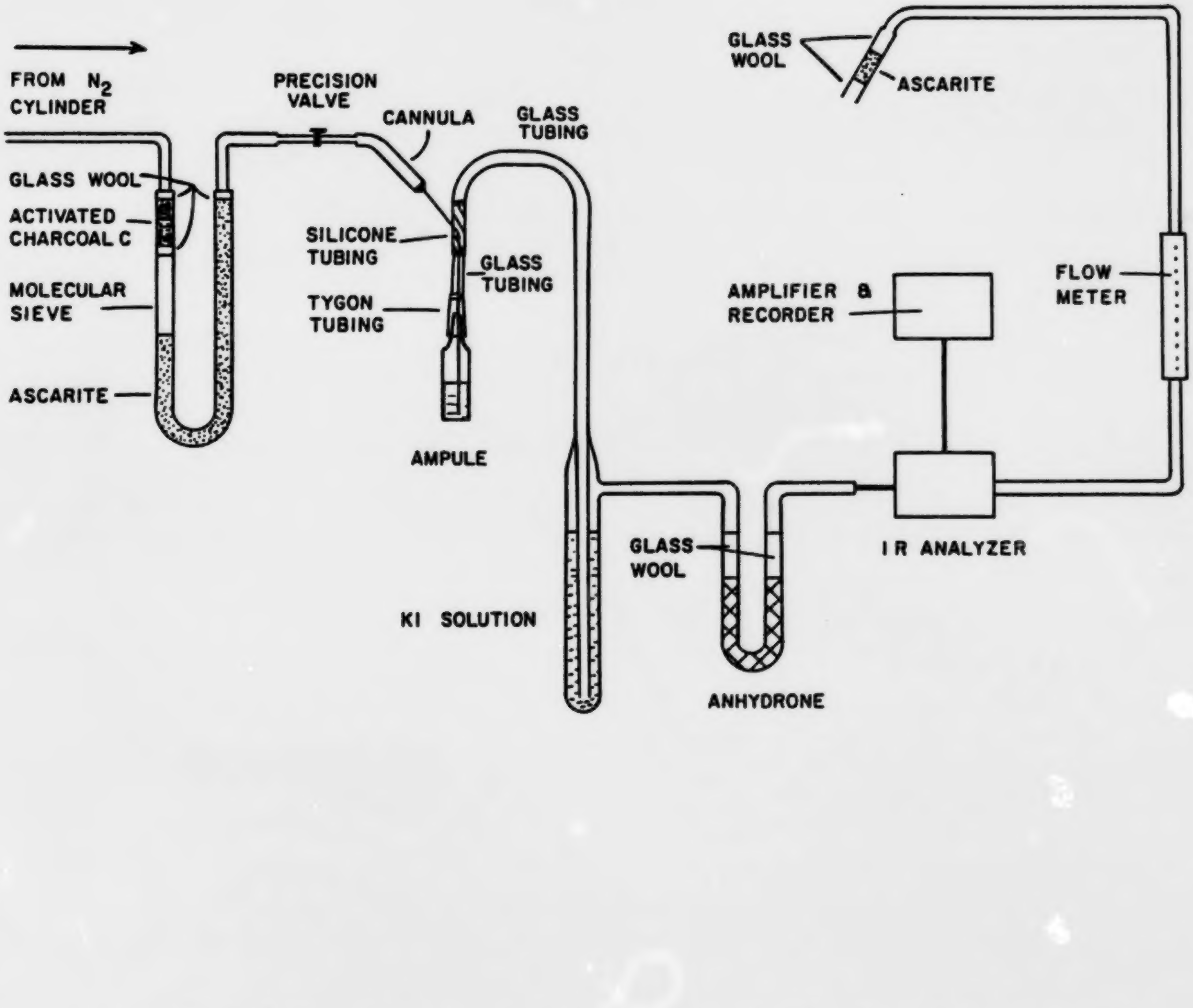
FROM N_2 CYLINDER
PRECISION VALVE
CANNULA
GLASS TUBING
GLASS WOOL
ACTIVATED CHARCOAL C
MOLECULAR SIEVE
ASCARITE
SILICONE TUBING
GLASS TUBING
TYGON TUBING
AMPULE
KI SOLUTION
GLASS WOOL
ANHYDRONE
GLASS WOOL
ASCARITE
AMPLIFIER & RECORDER
I R ANALYZER
FLOW METER

Figure III-3.3 Apparatus for measurement of CO_2 in sealed wet oxidation ampoules (Strickland and Parsons, 1972).

produced is not measured. The residue is then filtered or dried and combusted at high temperatures (650 - 1600°C) to determine TOC.

The technique of acid digestion of sediments, or carbonate dissolution, presents several concerns. A non-oxidizing acid must be chosen to avoid premature oxidation of organic carbon. Examples are H_3PO_4 (Froelich, 1980; Calvert, pers. comm; Iseki, pers. comm.), H_2SO_3 (Gibbs, 1977) and HCl (Plumb, 1981). With HCl, however, the production of Cl_2 via the formation of $CaCl_2$ during subsequent combustion of the sample, interferes with thermal conductivity detectors: Cl_2 traps are available but must be changed frequently (K. Iseki, pers. comm.).

A portion of the organic C is also soluble in acid. When the acid is applied to the sediment, care must be exercised to avoid losses of soluble organic carbon (SOC). If a permeable ceramic crucible is used to contain the sample, the sediment must be dried between the applications of acid (Calvert, pers. comm). If the sediment is filtered after acidification, 5-15% of organic C may be lost in carbonate-poor sediments, while > 45% may be lost in carbonate-rich/organic C-poor sediments (Froelich, 1980). The acid-soluble material is believed to be associated with foraminiferal or coccolith tests as surface or intercrystalline films (Heath et al., 1977). After acid digestion, TOC can be determined directly or determination can be made of Soluble Organic Carbon (SOC) and Residual Organic Carbon (ROC).

Direct determination methods remove inorganic C by acid treatment (Gibbs, 1977; Plumb, 1981 Iseki et al., 1984; Copin-Montegut and Copin-Montegut, 1972-73). The sample is then dried at 50-90°C, thereby avoiding filtration losses of inorganic C, and then oxidized in O_2 in an induction furnace. Gibbs (1977) determined optimum conditions of 1000 -1100°C for 30 seconds for complete combustion. Cupric oxide may also be used to assist in combustion. Suitable commercial instruments using thermal conductivity detectors are the Hewlett-Packard 185-B CHN analyzer (no longer in production), the LECO WR-12; LECO CHN/600, the Perkin-Elmer 240 C analyzer, Oceanography International Carbon Analyzer and the Carlo Erba 1106 CHN analyzer.

Direct determination procedures are relatively simple and fast. Wet chemistry and filtrations are not required. A further advantage is complete combustion of organic C at the temperatures used. Finally, the instruments cited will also determine Total Carbon and some have capability for hydrogen and nitrogen as well.

There are, however, some disadvantages with the method. Only TOC and TC can be determined directly. No confirming measurement for TIC can be made. Care must be exercised to avoid loss of SOC by frothing or leaching through permeable crucibles during acid treatment. Finally, acid-treated sediments which are dried and weighed will include nonstoichiometric calcium salts or excess acid (Froelich, 1980).

TOC can also be determined as the sum of Soluble Organic Carbon (SOC) and Residual Organic Carbon (ROC). In this approach, inorganic C is again converted to CO_2 - by treatment with H_3PO_4, sonication and N_2 bubbling - without measurement (Forelich, 1980). The spent acid supernant (SOC) is then separated from the residual material (ROC) by centrifugation and both are determined separately.

Soluble organic carbon (SOC) is determined by the modified Menzel and Vaccarro (1964) procedure for DOC. The solution is oxidized with $K_2S_2O_8$ in sealed ampoules (110°C for > 1 hour). An Oceanography International 0524 carbon analyzer measures the CO_2 evolved with a non-dispersive infrared detector. (Froelich, 1980; Mills and Quinn, 1979).

The residual organic carbon (ROC) is dry combusted using a Carlo Erba 1106 CHN/OS analyzer (O_2/Cr_2O_3 reactor at 1600°C) with GC separation of CO_2 and thermal conductivity detection.

The sum of the two measurements represents TOC. Total Carbon can also be determined by the Carlo Erba analyzer. The authors estimate the overall accuracy of this method to be ±2% relative and the precision with sediment with <90% $CaCO_3$ to be better than ±0.02% absolute TOC (Froelich, 1980). For example four replicate analyses of a sediment sample yielded a % TOC value of 0.240 ± 0.004 (standard deviation).

As a check for systematic errors, Froelich (1980) calculated TIC from TC-TOC and compared this to calcium values ($CaCO_3$ is the source of most Ca and carbonate in sediments).

The major advantage of this method is that inorganic C is completely removed without losing the acid-soluble fraction.

The method has the disadvantages that two major items of equipment are required and TIC is not determined directly. TOC values are the sum of two separate measurements. TOC results cannot therefore be checked by comparing C_{SUM} and C_{TOTAL}.

b) Acid Digestion and Total Dry Combustion

Inorganic C is converted to CO_2 by acidification in a closed system and the CO_2 produced is measured. (The apparatus is similar to that used in wet oxidation procedures). TOC is not determined directly, however. A second sub-sample is combusted at high temperatures to determine Total Carbon. TOC is therefore obtained by difference.

For TIC a 'reaction vessel' contains the dried sediment sample for acidification with HCl or H_3PO_4. The evolved CO_2 is swept by an O_2 stream through a condensor and H_2SO_4 scrubber to remove H_2O, an MnO_2 trap to remove SO_2 and a catalyst furnace to convert CO to CO_2 (Kolpack and Bell, 1968). Detection of CO_2 at this point can be done volumetrically - by measuring gas volume before and after absorbtion of CO_2 by a KOH solution (Kolpack and Bell, 1968) or gravimetrically, by absorbtion on ascarite (Calvert, pers. comm.). The Perkin Elmer 240C also has TIC capability, using thermal conductivity detection.

Total Carbon is determined on an untreated sample using dry combustion. The detection systems discussed above also interface with the furnace. TOC is calculated by difference: TOC = TC - TIC.

This technique is relatively simple to perform and the apparatus can also be used for TOC in an Acid Digestion/Dry Combustion method. Since TOC is determined by difference, the method may not be suitable for high carbonate sediments where TOC will be the difference between two large numbers and may fall within the error of the method (Calvert, pers. comm.).

The fact that two samples are required for each analysis may also be a problem where sample is limited or where sample inhomogeneity makes it difficult to obtain small replicate subsamples.

c) Differential Combustion

Differential combustion methods partition organic and inorganic carbon by combustion at different temperature. Several studies have indicated, however that separation by this method is not complete (Weliky et al., 1983; Froelich, 1980; Gibbs, 1977). Two approaches are discussed.

In the procedure described by Heath et al. (1977), organic C is first removed by dry combustion at 500°C for 2 hours. Evolved CO_2 is not measured. The same sample, which theoretically contains only inorganic C is then combusted at > 1200°C and evolved CO_2 is measured to give a value for TIC. A separate, untreated sample is then dry-combusted at > 1200°C to determine Total Carbon. TOC is determined by difference: TC - TIC = TOC. Heath et al. (1977) used a LECO Carbon Analyzer with thermal conductivity detection.

In the second method (Plumb, 1981) organic C is removed in a programmed microcombustion apparatus. The temperature is increased from 300°C to 650°C in 10 minutes and then held at 650°C for 20 minutes. The evolved CO_2 is trapped in ascarite and determined gravimetrically to give a TOC measurement. Total Carbon is also determined on a different sample using a temperature rise of 300°C to 950°C in 10 minutes with holding at 950°C for 20 minutes (CO_2 is again determined gravimetrically).

These methods have the advantage of simplicity. Minimal sample handling is required. Heath et al. (1979) found good agreement with an acid digestion LECO/dry combustion method for terrigenous and pelagic clays with low carbonate contents.

Gibbs (1977) however, found that a combustion temperature of 500°C was not effective for complete organic C removal. While a standard organic material (acetanilide) was completely combusted at 550°C, natural sediment samples required temperatures of 1000 - 1100°C. At 650°C, only 40% recovery was obtained for natural samples. Froelich (1980) also found 500°C combustion temperatures inadequate for organic material, recommending temperatures of >1000°C. For repeated pyrolysis of the sample at increasing temperatures, Froelich (1980) did not find a bimodal CO_2 release, which would be expected if C-CO_3 and C_{ORG} decomposed at different temperatures.

Weliky et al. (1983) also found TIC to be greatly underestimated by a Differential Combustion method. They observed thermal dissociation of carbonate material during the inorganic C removal at 500°C as $MnCO_3$, $MgCO_3$ and $FeCO_3$ will decompose at temperatures <500°C. Fe, Mn and Mg substituted in the $CaCO_3$ lattice will also cause lower dissociation temperatures.

3.2.4 Standard Reference Materials

Standard reference or calibration materials are shown in Table III-3.6. For inorganic carbon a natural standard (NBS Argillaceous Limestone) is available although several authors use pure carbonate materials.

For organic carbon natural standards are not available and pure compounds are used as shown. In order to calibrate an induction furnace, 1 g steel rings are combusted. At 1600°C, > 99% of the carbon is recovered (Calvert, pers. comm).

Two standard sediments (certified for total carbon only) are available from the National Research Council of Canada. They are MESS-1 having 2.99±.09 percent total carbon and BCSS-1 having 2.19±.09 percent total carbon.

TABLE III-3.6
STANDARD REFERENCE AND CALIBRATION MATERIALS

Carbon Phase	Standard Material/Source	Reference
$C_{INORGANIC}$	NBS Argillaceous Limestone	Calvert (pers. comm.)
	Reagent grade: $CaCO_3$	Weliky et al. (1983)
	$SrCO_3$	Weliky et al. (1983)
	Na_2CO_3	Copin-Montegut and Copin-Montegut (1972-73)
$C_{ORGANIC}$	NBS Acetanilide (C:N = 8)	Grasshoff et al. (1983) Froelich (1980)
	NBS Nicotinic Acid	Froelich (1980)
	Reagent grade: Adenine	Weliky et al. (1983)
	Sucrose	Weliky et al. (1983)
	Sucrose/SiO_2	Weliky et al. (1983)
	Tyrosine	Suzuki et al. (1983)
	Cystine	Suzuki et al. (1983)
	Glucose/NaCl	Suzuki et al. (1983)
	Oxalic acid Glucose	Copin - Montegut and Copin-Montegut (1972-73)
	LECO Stanrings (1g steel rings)	Calvert (pers. comm.)
C_{TOTAL}	MESS-1 BCSS-1	Russell, 1984

3.2.5 Summary

The choice of method for TOC will depend on available equipment and on the desired accuracy and precision. The partition of inorganic and organic C is the crucial element in TOC analysis. A variety of approaches have been developed (Table III-3.3) to deal with this problem but no standard method has, as yet, emerged. Specifications of the methods discussed are given in Table III-3.7. A summary of these methods with respective advantages and disadvantages is also given in Table III-3.8.

The simplest and least expensive method is the wet oxidation/titration procedure (Section 3.2.3.1a). The method suffers from incomplete oxidation, ranging from 70 - 90 % (EPS, 1979), from potential interferences by reduced species in the sediment and from standardization difficulties.

Instrumental wet oxidation methods (Section 3.2.3.1b) determine CO_2 directly and eliminate standardization and non-specificity errors but still suffer from incomplete oxidation of organic carbon.

In dry combustion methods, (Section 3.2.3.2) very high temperatures can be used to oxidize refractory organic material. Less elaborate systems are required than in the wet chemistry methods but the partition of inorganic and organic carbon may be a problem.

Differential combustion methods are not recommended due to the combustion of inorganic and organic carbon at overlapping temperatures. Methods which determine TC and TIC on separate samples and report TOC as TC-TIC are not generally applicable to sediment analysis. Large errors in TOC values may arise when two large numbers are subtracted and subsampling uncertainties may contribute significantly to the overall analysis uncertainty.

The choice of instrumental methods of detection will also depend on cost and study requirements. Thermal conductivity detectors, while the most expensive, have the greatest sensitivity and can also be used for H, N, O and S measurement.

TABLE III-3.7

SPECIFICATIONS FOR SEDIMENT TOC METHODS

METHOD	REFERENCE	SAMPLE SIZE	METHOD OF DETECTION	ESTIMATED SAMPLES/DAY
3.2.3.1 a)				
Wet Oxidation Titration	Gaudette *et al.* (1974), EPS (1980) Head *et al.* (1981)	.2 - .5 g	Titration	40 - 50
3.2.3.1 b)				
Wet Oxidation/ Instrumental	Weliky *et al.* (1983)	.05 - .10 g	Thermal Conductivity	20
	Suzuki *et al.* (1983)	Detection: 1-50 mg C	NDIR	15
	Mills and Quinn (1979)	.001 - .02 g	NDIR	20
3.2.3.2 a)				
Acid Digestion/ Dry Combustion	Gibbs (1977)	.005 - .05 g	Thermal Conductivity	40
	Plumb (1981)	.2 - .5 g	Gravimetric	40
	Froelich (1980)	.2 g	NDIR/Thermal	15
3.2.3.2 b)				
Acid Digestion/ Total Carbon Dry Combustion	Calvert (pers. comm.)	.5 g for TIC .2 g for TC	Gravimetric	50
	Kolpak and Bell (1968)	.2 - 1.0 g	Volumetric	50
3.2.3.2 c)				
Differential Combustion	Plumb (1981)	Detection: 25- 50 mg CO_2 (7- 14 mg C)	Gravimetric	50

TABLE III-3.8

COMPARISON OF METHODS FOR TOC IN SEDIMENTS

Summary of Method	Advantages	Disadvantages	Reference for Method
3.2.3.1 a) *Wet oxidation*/Titration	1. Simplicity. Suitable for unskilled personnel with minimal training. (40-50 samples can be run/day) 2. Lowest capital costs.	1. Not specific for organic C. Fe^{2+}, Mn^{2+}, S^{2-}, Cl^{-}, will interfere positively. 2. Organic C may be at different overall oxidation state than that used to standardize method. 3. Incomplete oxidation (70-90%) 4. TIC, TC not determined	Gaudette *et al.* (1983) EPS (1979) Head *et al.*(1981)
3.2.3.1 b) Acid digestion and *wet oxidation* in reaction vessel. CO_2 detection by LECO thermal conductivity apparatus. Confirming LECO dry combustion procedure.	1. Both TIC and TOC determined on same sample. 2. Acid soluble organic C retained in vessel. 3. TC determined, acts as a check to C_{SUM}. 4. Alternative detection gravimetric, volumetric, IR)	1. Relatively high capital cost of thermal conductivity detection.	Weliky *et al.* (1983)
Acid digestion and *wet oxidation* in reaction vessel. CO_2 absorbed by NaOH solution. Measurement by direct injection TOC analyzer Detection by NDIR.	1. Both TIC and TOC determined on same sample. 2. Acid-soluble organic C retained in vessel. 3. Cl and S do not interfere. 4. Adaptable by labs with direct injection TOC analyzer for water. 5. Ampoule sealing not required.	1. TC not determined, no check for for completeness of oxidation. 2. IR response non-linear. 3. Amount of absorbing solution must be optimized. 4. Inorganic C column deterioration due to injection of basic solution	Suzuki *et al.* (1983)
Wet oxidation in sealed ampoule. Measurement by TOC analyzer. Detection by NDIR.	1. Acid-soluble organic C retained. 2. Ampoules processed in batches, can be sealed and stored. 3. Adaptable by laboratories with DOC capabilities.	1. TIC, TC not determined; no check for completeness of oxidation 2. IR response non-linear. 3. Ampoule sealing technique requires skill and practice.	Mills and Quinn (1979)
3.2.3.2.a) Acid digestion to remove inorganic C. *Dry combustion* of organic C. Detection by thermal conductivity, gravimetric or volumetric.	1. Procedure relatively simple. (40 samples can be run/day) 2. Complete combustion occurs at high temperatures used (< 1000°C). 3. TC, hydrogen, nitrogen can also be determined by CHN analyzer. (Gravimetric, volumetric detection possible at lower capital cost).	1. TIC is not measured. Therefore no confirmatory check for C_{SUM}. 2. Acid-soluble C can be lost through frothing, leaching through permeable crucible. 3. Acid-treated, dried sediments will contain nonstoichiometric Ca salts and excess acid.	Plumb (1981) Gibbs (1977)
Acid digestion to remove inorganic C. SOC and ROC separated by centrifugation. SOC det. by DOC procedure, ROC by *dry combustion*.	1. Acid-soluble fraction accounted for. (TOC is sum of SOC and ROC). 2. Filtration and drying not required.	1. TIC is not measured. Therefore no confirmatory check for C_{SUM}. 2. TOC analyzer and CHN analyzer required.	Froelich (1980)
3.2.3.2.b) Acid digestion of inorganic C. TIC measured gravimetrically volumetrically or by thermal conductivity. TC det. by *dry combustion*.	1. Procedure relatively simple. (50 samples can be run/day) 2. Apparatus can also be used in method 3.2.3.2 a (for high carbonate sediments).	1. TOC determined by difference. not suitable for high carbonate sediments as TOC value may fall within error of method (See Ad. 2) 2. Two samples are required.	Calvert (pers. comm.) Kolpack & Bell (1968) Perkin-Elmer 240C elemental analyzer (1984)
3.2.3.2.c) *Differential combustion* of organic C at 500 - 650°C and inorganic C at 950 - 1600°C.	1. Procedure relatively simple. Minimal handling. (50 samples can be run/day).	1. Organic C/Inorganic C partition 2. Organic C requires combustion temperature of >1000°C. 3. Thermal dissociation of carbonates begins at < 500°C. 4. Two samples are required.	Plumb (1981) Heath *et al.* (1977)

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3.2.7 Personal Communications

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3.3 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) measurements are carried out as an indication of organic material in water and sediments. COD can be procedurally-defined as a "measure of the O_2 equivalent of organic matter susceptible to oxidation by a strong chemical oxidant" (APHA, AWWA, WPCF, 1980). The method as applied to sediments in particular, suffers from non-specificity and incomplete reaction.

The sediment sample is digested with a strong oxidizing agent at low pH, the amount of oxidant consumed is measured and is expressed as an equivalent amount of O_2. The standard method is a reflux procedure employing potassium dichromate ($K_2Cr_2O_7$) as an oxidizing agent and Ag_2SO_4 as a catalyst. Excess $K_2Cr_2O_7$ is then titrated with ferrous ammonium suplfate (FAS: $Fe\ (NH_4)_2(SO_4)_2 \cdot 6H_2O$).

It is interesting to note that a computerized literature search of CISTI (Canadian Institute for Scientific & Technical Information) abstracts revealed a majority of references to COD in sediments in Japanese and German publications. Very few examples for North American journals were found. Some examples have therefore been drawn from literature on COD in water, as the dichromate oxidation procedure is very similar.

COD is sometimes used rather than the relatively time-consuming Biochemical Oxygen Demand (BOD) test (Moore, et al., 1949). BOD is also a procedurally-defined measure of organic material, specifically the "amount of O_2 consumed by microbial organisms while assimilating and oxidizing the organic material present" (Plumb, 1981). The relationship between COD and BOD, however, will depend on sediment characteristics and cannot be generalized. In certain applications (wastewater, effluent, pollution and metal toxicity studies) the COD:BOD ratio is used to give information on the biodegradability of the organic material present (Aziz and Tebbutt, 1980; Bridie et al., 1979; Ajmal et al., 1983). COD has also been empirically related to TOC (Total Organic Carbon) and TOM (Total Organic Matter) for specific sources (Schreiber and McGregor, 1979; APHA, AWWA, WPCF, 1980). These parameters, however, have been found to be poorly correlated with COD where sample composition is subject to change (i.e., microbial degradation of biologically susceptible compounds, Aziz and Tebbutt, 1980).

The chemical component of Sediment Oxygen Demand (SOD) is also known as chemical oxygen demand. SOD is a measure of the influence of sediments on dissolved oxygen in a parcel of water (Wang, 1980) and may account for a significant proportion of oxygen consumption in the overlying waters (up to 80%) (Adams et al., 1982; Edberg and Hofsten, 1973). SOD can be measured with an in situ device or on laboratory-incubated samples (Bowman and Delfino, 1980; Plumb, 1981; Markert et al., 1983; Adams et al., 1982; Sonzogni et al., 1977). The total oxygen demand can be fractionated into biological and chemical components by the use of biological toxicants (Wang, 1980; Barcelona, 1983).

The oxidation efficiency for water samples in COD procedures has been estimated at 85-95% (Baumann, 1974). No information is available for sediment samples. Volatile compounds are difficult to oxidize as they are present in the vapor space and may not come in contact with the oxidizing agent (APHA, AWWA, WPCF, 1980). The use of Ag_2SO_4 as a catalyst, assists in the oxidation of straight chain aliphatics and organic polymers such as cellulose (APHA, AWWA, WPCF, 1980; EPS, 1979; Moore et al., 1951).

A serious limitation of the COD method is its non-specificity. Reduced cations and anions such as Fe^{2+}, Mn^{2+}, S^{2-}, NO_2^- and Cl^- will interfere positively with the dichromate method (Plumb, 1981).

3.3.1 Storage

According to the EPA/Corps of Engineers method (Plumb, 1981), sediment samples are stored in clean (see Section 3.2.1) plastic or glass containers, minimizing air contact and storage headspace to prevent air oxidation. Storage of moist samples at 4°C is recommended, with a time limit of one week.

3.3.2 Pre-treatment

Pre-treatment for the dichromate oxidation method consists of homogenizing the field-moist sample. The wet sediment is used directly for refluxing (sample size 0.5 - 2.0 grams). The elimination of air oxidation is the rationale for this approach. Weighing of a separate dried sample is required for final dry weight calculations.

3.3.3 Method of Analysis

For COD in water, dichromate titration methods are available in several reference manuals with minor variations in procedure (EPS, 1979; APHA, AWWA, WPCF, 1980; Plumb, 1981; Inland Waters Directorate, 1979; Benda et al., 1978; ASTM D1252-67 (reapproved 1974)). The Environmental Protection Agency (Plumb, 1981) has also outlined a method for COD in sediments. This procedure is essentially the same as that for aqueous samples. Interferences in sediments may be greater than in aqueous samples, however, due to higher concentrations of reduced inorganic species (Plumb, 1981).

All procedures contain the following elements:

1) Oxidation of organic material with 0.25N $K_2Cr_2O_7$ or $KMnO_4$ (Moore et al., 1949, 1951; Medalia, 1951). Permanganate gives consistently lower levels of COD (Mrkva, 1983).

2) Catalysis of oxidation with Ag_2SO_4 (Moore et al., 1951). Eissa (1983) also used $NiSO_4$ and CuO as catalysts.

3) Complexation of Cl^- (and other halides) with $HgSO_4$ at a weight ratio of 10:1 $HgSO_4$:Cl^-. This has two functions: to avoid the precipitation of AgCl and co-precipitation of otherwise oxidizable material (APHA, AWWA, WPCF, 1980) and secondly, to prevent positive interference by the oxidation of Cl^- to Cl_2 (Dobbs and Williams, 1963).

4) Prevention of NO_2^- oxidation with the addition of sulfamic acid.

5) Reflux of mixture for 2 hours.

6) Titration of excess dichromate with ferrous ammonium sulfate (FAS).

The dichromate method for COD is similar to the "Dichromate wet oxidation/titration" method used to determine total organic carbon (TOC) and suffers from the same limitations (Section 3.2.3.1a). A comparison is given in Table III-3.9.

Canelli et al. (1976) found the reflux procedure to be unneccessarily combersome to perform. For COD in water they developed a non-reflux method (140°C) with colorimetric determination of excess dichromate. The results are comparable to the standard reflux procedure. One hundred ninety-two samples can be processed simultaneously. The method was not applied to sediments.

TABLE III-3.9

COMPARISON OF COD AND TOC METHODS FOR SEDIMENTS

	COD	TOC
Oxidant	0.25 N $K_2Cr_2O_7$	1N $K_2Cr_2O_7$
Catalyst	Ag_2SO_4	-
Acid Medium	H_2SO_4	H_2SO_4
Reaction Conditions	Reflux, 2 hours	Exothermic, 30 minutes
Correction for Cl^-	1. Complex with $HgSO_4$ 2. Correct by standard curve.	1. Precipitate with Ag_2SO_4 2. Calculated correction within error of method. (Gaudette et al., 1974)
Determination	Titration with FAS	Titration with FAS
Result	COD (mg g^{-1} O_2 equivalent)	TOC (%)
References	Plumb (1981)	Gaudette et al. (1974) EPS (1979)

There are several potential interferences and sources of error with the COD method. These are outlined below with possible corrections and preventative steps.

1) Incomplete oxidation of refractory organic material. In water, Baumann (1974) estimates 85-95% oxidation. For a selection of individual compounds, Bridie et al. (1979) show COD with a range of 31-102% of the theoretical oxygen demand.

2) Interference by Cl^- and other halide ions. For the standard dichromate reflux method in water, the limit for an acceptable Cl^- concentration ranges from 1000 - 2000 mgL^{-1} (EPS, 1979). Above this limit, $HgSO_4$ complexation of Cl^- is no longer completely effective or predictable (Canelli et al., 1976). This concentration of Cl^- corresponds to 20-40 mg of Cl^- in a 20 mL sample. A 2 gram aliquot of marine sediment containing 50% water with a salinity of 35%, would contain 21 mg of Cl^-. Most marine sediments should therefore have Cl^- concentrations within this limitation.

Baumann (1974) suggests direct measurement of evolved Cl_2. He found that at 10,000 mg L^{-1} Cl^- (corresponding to a salinity of 16.6 %) a 20:1 ratio of $HgSO_4$:Cl was ineffective, as some Cl^- was still oxidized. He therefore suggests trapping the evolved Cl_2 in an acidic KI solution and titration with sodium thiosulfate (NaS_2O_7).

3) Interference by reduced inorganic species Fe^{2+}, Mn^{2+} and S^{2-}. The APHA, AWWA, WPCF (1980) Manual assumes stoichiometric oxidation. If the initial concentration is known, then a correction can be applied. This however involves additional analyses.

4) Interference by nitrite (NO_2^-) with a COD of 1.1 mg O_2/mg NO_2^- - N (APHA, AWWA, WPCF, 1980). This can be eliminated by the addition of sulfamic acid (10 mg/mg NO_2^- - N).

5) Interference by SO_2 in industrial waste. Watts and Adams (1983) suggest the addition of Mn^{2+} with oxygenation to catalyze the oxidation of SO_2 to SO_4^{2-}, prior to COD analysis. Mn^{2+} is oxidized to Mn^{4+} or higher states of oxidation.

6) The spent reaction mixture contains toxic Hg^{2+} and Ag^+. These can be removed using selective ion-exchange resins or precipitated as sulfides (Benda et al., 1978).

3.3.4 Standard Materials

The APHA, AWWA, WPCF (1980) manual, recommends potassium hydrogen phthalate as a standard compound for COD in water. Bridie et al., (1979) give theoretical oxygen demands for a variety of industrial organics, along with experimental COD and BOD values.

3.3.5 Summary

The standard method for COD in both water and sediments is a dichromate reflux. This procedure has been applied as an indicator of organic material and as a monitoring parameter related to BOD, TOC and TOM. However, the method suffers from non-specificity which complicates the relationship with sediment organic carbon. Reduced species in sediments (Fe^{2+}, Mn^{2+}, S^{2-}, Cl^-, NO_2^-) will produce a positive interference. The oxidation of organic material may also be incomplete. Plumb (1981) has recommended that the COD of sediments not be used as an organic indicator.

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Moore, W.A., R.C. Kroner and C.C. Ruchhoft, 1949. Dichromate reflux method for determination of oxygen consumed. Anal. Chem. 21: 953-957.

Moore, W.A., F.J. Ludzack and C.C. Ruchhoft, 1951. Determination of oxygen-consumed values of organic wastes. A comparative study of methods. Anal. Chem. 23: 1297-1300.

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Schreiber, J.D. and K.C. McGregor, 1979. The transport and oxygen demand of organic carbon released to runoff from crop residues. Prog. Wat. Tech. 11: 253-261.

Sonzogni, W.C., D.P. Larsen, K.W. Malveg and M.D. Schuldt, 1977. Use of large submerged chambers to measure sediment - water interactions. Water Res. 11: 461-464.

Wang, W., 1980. Fractionation of Sediment Oxygen Demand. Water Res. 14: 603-612.

Watts, R.J. and V.D. Adams, 1983. The elimination of sulfur dioxide interference in the low level Chemical Oxygen Demand analysis. Water Res. 17: 715-718.

3.4 Petroleum Hydrocarbons

3.4.1 Introduction

There are numerous biogenic, diagenetic and anthropogenic sources of hydrocarbons in the marine environment. Anthropogenic sources include inputs from petroleum exploration, production and transportation activities (including both normal operations and accidental spills) as well as from sewage outfalls, discharge of industrial effluents and fallout from the atmosphere. The most important anthropogenic sources of petroleum hydrocarbons in the arctic environment are spillages of refined oil in populated areas (Horowitz et al., 1978) and offshore drilling activities (Thomas et al., 1983). Petroleum hydrocarbons entering the marine environment have various fates. The volatile hydrocarbon components are lost to the atmosphere. Some hydrocarbons dissolve in the water column, while poorly soluble components are deposited in surface sediments (Farrington and Tripp, 1975, 1977; Hilpert et al., 1978) where they may persist for years. A portion of the hydrocarbons introduced into the marine environment may also accumulate in lipid-rich marine organisms (Smith et al., 1984; Lee et al., 1981). Mussels for instance, have been shown to concentrate aromatic hydrocarbons from seawater by a factor of up to ten thousand (Ehrhardt and Heinemann, 1975).

Biogenic hydrocarbons in the marine environment include normal and branched chain alkanes in the range of one (C_1, methane) to thirty-three (C_{33}) carbon atoms. Compounds containing an odd number of carbon atoms predominate in marine biotic systems and the isoprenoid pristane (C_{19}) is a major component (Blumer, 1957). Alkenes are also abundant compounds from all trophic levels. After deposition in sediment, biogenic molecules may be altered by microbial and chemical processes (diagenesis) to yield a variety of compounds including aliphatic hydrocarbons, cycloalkenes and polycyclic aromatic hydrocarbons (PAHs), some of which are also found in petroleum and other anthropogenic sources (Boehm, 1981; Wakeham et al., 1981).

Petroleum and petroleum products are a complex mixture of many different hydrocarbon compounds covering a wider boiling range than those of biogenic origin. The main classes of petroleum compounds of environmental interest are saturates and aromatics. The saturates consist of normal alkanes, branched alkanes, cycloalkanes and isoprenoid alkanes. The ratio of odd to even carbon number alkanes in petroleum hydrocarbons is close to unity, and there are generally no alkenes. The aromatic fraction contains aromatic and alkylated aromatic compounds of up to six aromatic rings. Polycyclic aromatic hydrocarbons (PAHs) and alkylated derivatives comprise up to 15% of petroleum products. Commonly determined PAH compounds are listed in Table III-3.10.

Methods used for the analysis of total hydrocarbons in sediment and biota samples are not able to distinguish hydrocarbons from biogenic, diagenetic and anthropogenic sources. However, determinations such as the pristane/phytane ratio or odd/even predominance (OEP) of alkanes can be used to estimate the relative importance of biogenic and petroleum inputs (Boehm, 1981). Pristane, a C_{19} isoprenoid, is rarely found in biogenic inputs, while phytane, a C_{20} isoprenoid, is a dominant component of biogenic inputs. The pristane/phytane ratio is close to unity in petroleum hydrocarbons while it approaches zero in biogenic hydrocarbons. Weathered petroleum hydrocarbons can be distinguished from fresh inputs by monitoring the normal alkane to isoprenoid ratio which is much larger than unity in fresh petroleum samples but decreases as biodegradation of the sample proceeds (Boehm, 1981). In petroleum there is an approximate equal distribution of adjacent alkanes with an odd and even number of carbon atoms. The alkanes of recent biological origin, however, have a predominance of compounds with an odd number of carbon atoms.

The analysis of petroleum hydrocarbons in biota or sediment samples is a three-step procedure. The hydrocarbons are separated from the biota or sediment matrix, usually by a solvent extraction procedure. The extract solution is then cleaned to remove interfering co-extracted material and the hydrocarbons fractionated into groups to facilitate their determination. Cleanup and fractionation are usually accomplished by adsorption chromatography but other methods are also discussed. The final step is the determination of the hydrocarbons in the extract, either as a total hydrocarbon concentration or as concentrations of individual components. Gas chromatography with flame ionization or mass spectrometric detection is the quantification technique most commonly employed.

Concentrations of petroleum hydrocarbons reported in arctic sediment and biota samples are summarized in Table III-3.11.

3.4.1.1 Scope

The effect of petroleum hydrocarbons in environmental systems is currently a topic of great interest. The scientific literature contains many descriptions of analysis procedures used for determining hydrocarbons in environmental samples. Because of the abundance of information available, this report presents only a brief

TABLE III-3.10

PAH Compounds Found in Petroleum

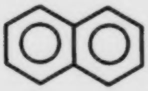
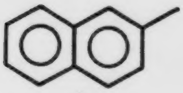
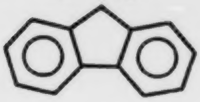
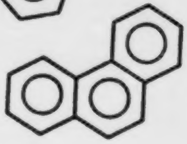
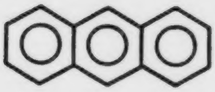
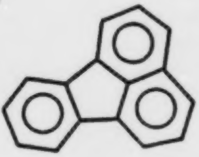
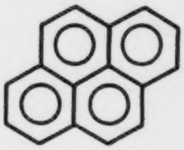
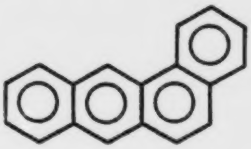
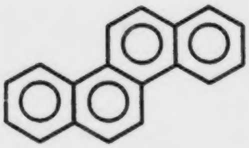
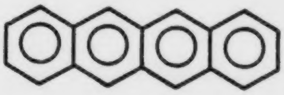
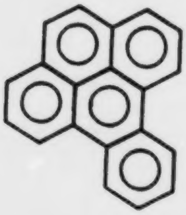
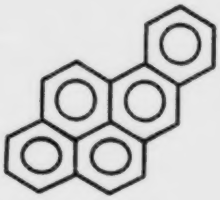
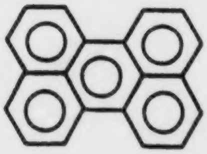
Molecular Structure	Name	Formula
	Naphthalene	$C_{10}H_8$
	2-methylnaphthalene	$C_{11}H_{10}$
	Fluorene	$C_{13}H_9$
	Phenanthrene	$C_{14}H_{10}$
	Anthracene	$C_{14}H_{10}$
	Fluoranthene	$C_{16}H_{10}$
	Pyrene	$C_{16}H_{10}$
	Benz(a)anthracene	$C_{18}H_{12}$
	Chrysene (1,2-benzophenanthrene)	$C_{18}H_{12}$
	Naphthacene	$C_{18}H_{12}$
	Benzo(e)pyrene	$C_{20}H_{12}$
	Benzo(a)pyrene (3,4-benzpyrene)	$C_{20}H_{12}$
	Perylene	$C_{20}H_{12}$

TABLE III-3.11

HYDROCARBON LEVELS IN ARCTIC SEDIMENT AND BIOTA

Sample Type	Location	Hydrocarbon Types	Mean Concentration	Reference
Sediment	Issungnak	selected PAHs	639 $ng \cdot g^{-1}$	Erickson et al., 1983
Sediment	Tarsiut	total PAHs	1,200 $ng \cdot g^{-1}$	Thomas, 1982
Sediment	Southern Beaufort Sea	selected PAHs	1,242 $ng \cdot g^{-1}$	Wong et al., 1976
Sediment	Southern Beaufort Sea	selected PAHs	541 $ng \cdot g^{-1}$	Wong et al., 1976
Sediment	Alaskan Beaufort Sea (20 locations)	total saturates	0.1 - 12.5 $\mu g \cdot g^{-1}$	Shaw, 1978
Sediment	Alaskan Beaufort Sea (20 locations)	total unsaturates	0.01 - 7.3 $\mu g \cdot g^{-1}$	Shaw, 1978
Fish	Prudhoe Bay	saturates	0.9 - 10 $ng \cdot g^{-1}$	Feder et al., 1976
Fish	Prudhoe Bay	aromatics	0.5 - 235.1 $ng \cdot g^{-1}$	Feder et al., 1976
Fish	Beaufort Sea	total PAHs	23 $ng \cdot g^{-1}$	Thomas, 1982

description of the methods most commonly used for marine sediment and biota samples. More detailed information can be obtained from the literature cited in the following sections.

3.4.2 Sample Handling

Hydrocarbon compounds are common in the environment and in the laboratory. Thus caution must be exercised to avoid contamination of field samples at all stages of collection, preservation and sample storage. The exclusion of field (shipboard) contaminants is particularly difficult when sampling from a research vessel (Clark and Brown, 1977). Extreme care must be taken to avoid sample contamination from the sampling device, from the ship and ship's discharges and from oil in the water column. Lubricating oils and greases, fuel oil and engine room wastes are among the most easily introduced materials. Sources of laboratory contaminants include lubricants, plasticizers, rubber and reagent grade chemicals and solvents.

The size of sample required for analysis is dictated by the expected level of hydrocarbons and by the sensitivity of the analysis technique employed. With current analytical techniques, sediment and biota samples in the range of five to ten grams dry weight are adequate for the levels of hydrocarbons commonly encountered in marine sediments and biota (Wong and Williams, 1980; Boehm, 1981).

Clark and Brown (1977) have discussed in detail the collection of sediment and biota samples for hydrocarbon analysis. Glass bottles with screw-on lids are suitable for storing samples. The wax-coated liner of the lid should be replaced with aluminum foil or with solvent-extracted Teflon. Aluminum or stainless steel containers may also be used. All other containers such as those made of plastic, rubber, fiberglass or unglazed ceramic are unsuitable. All containers and glassware should be cleaned with detergent and rinsed with acetone and then with a second solvent such as pentane, hexane or methylene chloride or baked at 350°C overnight. Biota samples from oil spill areas are sometimes rinsed in hexane or cyclohexane to remove surface hydrocarbons before being placed in containers or wrapped in aluminum foil (Fong, 1976; Smith et al., 1984). This practice should be unnecessary for benthic species or species from non-spill areas and may even result in partial extraction of hydrocarbons from the specimens.

Samples to be analyzed for low molecular weight hydrocarbons require special handling. Sediment samples should fill the sample container completely and be frozen immediately (Boehm, 1981). Alternatively, some authors recommend transferring sediments to containers holding "poisoned" (e.g. sodium azide, potassium cyanide) hydrocarbon-free seawater, flushing the container headspace with helium or nitrogen and inverting the container at near-freezing temperatures (Bernard et al., 1978; Horowitz et al., 1978) Others have found that sodium azide is not an acceptable poisoning agent for samples intended for analysis of low molecular weight hydrocarbons (Levy, pers. comm.). Biota samples should be tightly wrapped in aluminum foil until subsampled for purgeable organics (Boehm, 1981).

3.4.2.1 Sample Storage

Volatilization of hydrocarbon components and microbial and photochemical oxidation of organic matter in samples are the primary concerns to be addressed in post-sampling storage of materials for hydrocarbon analysis. Samples should be frozen at -10°C to -20°C immediately after they are collected if extraction and analysis cannot be started immediately (Clark and Brown, 1977). The effect of long-term (months to years) storage on sample integrity is unknown. Medeiros and Farrington (1974), however, determined that analytical results were unchanged after eighteen months of storage of oil-spiked cod liver lipid extract. Wise et al.(1980) concluded from the results of an interlaboraory comparison of trace hydrocarbon determinations in mussels that time of storage may be a contributing parameter to the variability of reported results.

3.4.3 Sample Pretreatment

Sediment samples can be pre-dried prior to extraction of hydrocarbons. Pre-drying is required for extraction methods employing a non-polar solvent but not for methods employing polar solvents such as methanol or methanol mixtures (Boehm, 1981). Pre-drying has been achieved by physical methods such as freeze-drying, air-drying or oven-drying (Overton and Laseter, 1980; Hilpert et al., 1978). Wong and Williams (1980) reported a sixteen percent loss of hydrocarbons from sediments oven-dried at 45°C for two days while Hilpert et al. (1978) reported that some volatile hydrocarbons (up to C_{20}) were lost from sediments during freeze-drying. Therefore if pre-drying of the sediments is required, de-watering with methanol is the preferred method. The methanol wash is saved and combined with the subsequent sample extract to avoid loss of hydrocarbon material (Brown et al., 1980).

There is little information available in the literature to determine whether or not depuration of biota is required prior to analysis. If the entire specimen is to be analyzed, depuration may be carried out to ensure that the gut contents do not bias the tissue analysis results. The appropriate depuration time is not well defined in the literature and care must be exercised to ensure that contaminants are not also being lost from the tissues. For example, Lee (1977) has calculated that the half-life of petroleum hydrocarbons in mussels and oysters can be as short as 3.5 and 2 days respectively. Depuration should be started as soon after sample collection as possible.

3.4.4 Extraction of High Molecular Weight Hydrocarbons

An extraction step is required to quantitatively isolate the non-volatile hydrocarbons from the sample matrix (sediment or biota). Several methods are commonly used but no standard method exists for either sediment or biota. The ease of extraction of hydrocarbons from a sediment matrix will vary with the type of sample and how the hydrocarbons are bound to the sediment particles. A rigorous extraction procedure may be required to extract low to moderate levels of hydrocarbons from uncontaminated sediment samples, while simpler techniques may suffice for samples contaminated by oil spills. Hydrocarbons present in marine biota include those loosely bound to the tissue matrix and those occurring intracellularly. The high natural lipid content of many organisms and some sediments presents additional extraction and other analytical problems.

3.4.4.1 Sediments

A summary of the methods used to extract hdyrocarbon material from sediment samples is given in Table III-3.12. Many of these are solvent extraction methods but other techniques such as steam distillation (Veith and Kiwus, 1977; Bellar et al., 1980) and headspace gas stripping (May et al., 1975) have been employed.

Most of the solvent extraction methods involve the combined use of polar and non-polar solvents to effect an efficient extraction of organic matter from the sediment (Brown et al., 1980; Farrington and Trip, 1975; Lake et al., 1980). Non-polar solvent (e.g., hexane) extractions are also successful if the sediment has been pre-dried by some physical (freeze-drying or air-drying) or chemical (methanol extraction) technique (Boehm, 1981). Four types of solvent extractions are employed:

i) soxhlet extractions (Farrington and Tripp, 1975; Hites et al., 1980),
ii) reflux extractions (Lake et al., 1980),

TABLE III-3.12

EXTRACTION METHODS FOR HYDROCARBONS IN SEDIMENTS

EXTRACTION TECHNIQUE	SOLVENT	HYDROCARBON TYPE	REFERENCE
soxhlet	methanol:methylene chloride	PAHs	Bates et al., 1984
soxhlet	methylene chloride	saturated hydrocarbons, PAHs	Giger and Schaffner, 1978
soxhlet	benzene or acetonitrile:benzene or acetone:cyclohexane:methanol	PAHs	Haddock et al., 1983
soxhlet	1:1 methanol:benzene	PAHs	Lake et al., 1980
soxhlet	methanol	PAHs	Plumb, 1981
soxhlet	carbon tetrachloride	saturated hydrocarbons, PAHs	Wong and Williams, 1980
soxhlet	chloroform	saturated hydrocarbons, PAHs	Wong and Williams, 1980
1. soxhlet 2. soxhlet	methanol methanol:benzene	PAHs	Hites et al., 1980
1. digestion 2. soxhlet	methanolic potassium hydroxide methanol	saturated hydrocarbons, PAHs	Wong and Williams, 1980
1. soxhlet 2. reflux	1:1 benzene:methanol ethanolic potassium hydroxide	saturated hydrocarbons	Farrington and Tripp, 1975
reflux	ethanolic potassium hydroxide;benzene	saturated hydrocarbons	Farrington and Tripp, 1975
reflux	methylene chloride	PAHs	Lake et al., 1980
reflux	methanolic potassium hydroxide; toluene (or benzene)	PAHs	Farrington and Quinn, 1973; Lake et al., 1980
reflux	ethanolic potassium hydroxide; solvent partitioning	PAHs	Plumb, 1981; Cretney et al., 1980
reflux	0.5 m sodium hydroxide	PAHs	Lake et al., 1980
1. reflux 2. reflux	hexane benzene	PAHs	Overton and Laseter, 1980
1. reflux 2. digestion	methanol:toluene ethanolic potassium hydroxide	PAHs	Van Vleet and Quinn, 1978
shaking	1:4 acetone:pentane	saturated hydrocarbons, PAHs	Friocourt et al., 1981
shaker	artifical sea water	saturated hydrocarbons, PAHs	Templeton and Chasteen, 1980
ball mill tumbler	methylene chloride	PAHs	Lake et al., 1980
ball mill tumbler	2:1 methylene chloride:methanol	saturated hydrocarbons, PAHs	Brown et al., 1980
sonication	2:1 methylene chloride:methanol	PAHs	Albaiges and Grimalt, 1982
sonication	cyclohexane	PAHs	Tan, 1979
headspace sampling/ coupled-column liquid chromatography		saturated hydrocarbons, PAHs	May et al., 1975

iii) ambient temperature extractions using a shaker table or ball-mill tumbler (Brown et al., 1980) and

iv) ultrasonic extractions (Tan, 1979).

Saponification by alkali digestion is sometimes used in conjunction with solvent extraction to remove "tightly bound" hydrocarbon material from the sediment. Farrington and Tripp (1975) described a method in which solvent extraction is preceeded by saponification by an alkali digestion while Van Vleet and Quinn (1978) first reflux the sediment in methanol-toluene solvent and then use an alkali digestion step to extract additional hydrocarbon material. Farrington and Quinn (1973) reported a simultaneous saponification/extraction by using a methanolic potassium hydroxide-toluene reflux to remove hydrocarbons from sediment. Saponification will cause chemical transformation of many chlorinated hydrocarbons, particularly DDT, DDD and DDE (Farrington and Tripp, 1975; Boehm, 1981) and is therefore not advised if the same extract solution is to be analyzed for both petroleum hydrocarbons and chlorinated hydrocarbons.

Two non-solvent extraction methods have been used for hydrocarbons in sediments. May et al. (1975) employed dynamic headspace sampling and GC analysis of hydrocarbons volatilized at 70°C for four hours followed by coupled-column liquid chromatographic analysis of the non-volatile components. Veith and Kiwus (1977) extracted hydrocarbons by exhaustive steam distillation.

Several authors have undertaken comparisons of specific sediment extraction techniques. Farrington and Tripp (1975) compared the results of the extraction of a marine sediment by:

i) twenty-four hour soxhlet extraction with benzene:methanol followed by saponification with methanolic potassium hydroxide, and

ii) simultaneous extraction/saponification by three hour reflux with benzene-methanolic potassium hydroxide.

They reported no significant difference in either the composition of the aliphatic fraction or in the total weight of hydrocarbon material extracted by the two methods. They did not examine the composition of the aromatic fractions extracted.

Wong and Williams (1980) compared three extraction methods for hydrocarbons in marine sediments:

i) saponification by methanolic potassium hydroxide followed by soxhlet extraction with methanol,
ii) soxhlet extraction with chloroform and
iii) soxhlet extraction with methanol.

Their results indicate that the three methods give comparable recoveries of hydrocarbons from dried sediments. The results of the saponification/methanol extraction method are the most precise. The chloroform and carbon tetrachloride extraction methods gave variable results on wet sediments.

Lake et al. (1980) compared seven extraction procedures including soxhlet, tumbler, reflux and alkali digestion. They concluded that the soxhlet extraction method with benzene-methanol and the reflux method with methylene chloride showed no statistical differences in their efficiency of extracting polycyclic aromatic material from marine sediment, while the ball-mill tumbling extraction method (with ambient temperature methylene chloride) was only 72% as efficient.

Brown et al. (1980) compared the ball-mill tumbling extraction method using various ambient temperature solvent systems to three boiling solvent extraction methods:

i) reflux with ethanolic potassium hydroxide,
ii) soxhlet extraction with benzene:methanol, and
iii) soxhlet extraction with methylene chloride:methanol.

They reported that the ball-mill tumbler method using 2:1 methylene chloride:methanol recovered hydrocarbons from a marine sediment as efficiently as did the three boiling solvent techniques.

These results suggest that, with the proper choice of solvent, any of the widely used solvent extraction techniques (soxhlet, reflux or tumbler) will efficiently extract hydrocarbons from sediment samples. Soxhlet extraction is the most exhaustive technique but also the most time consuming (seventy-two hours in some cases versus three hours refluxing). The ball-mill tumbling method is also time consuming (forty hours) but does not require boiling solvents, running water or expensive glassware and allows routine processing of large numbers of samples.

3.4.4.2 Biota

Several methods have been utilized to extract petroleum hydrocarbons from marine organisms. These are outlined in Table III-3.13. Homogenized wet tissues can be saponified by alkali digestion using ethanolic potassium hydroxide; the liberated hydrocarbons are then partitioned into an organic solvent such as diethyl ether, pentane, hexane or iso-octane (Warner, 1976; Cretney et al., 1980; Dunn, 1976). Hydrocarbons can be separated from dry tissue samples by successive soxhlet extractions using first methanol and then a non-polar solvent such as hexane, benzene or methylene chloride (Ehrhardt and Heinneman, 1975). Tissue samples have also been extracted by steam distillation (Vale et al., 1970; Ackman and Noble, 1973; Veith and Kiwus, 1977). Simultaneous sample homogenization and extraction can be achieved by blending the sample with anhydrous sodium sulfate and a non-polar solvent followed by phase separation of the organic layer (Gay et al., 1980). Extraction of hydrocarbons from tissue samples by refluxing with hexane followed by benzene has also been reported (Lawler et al., 1978).

Absolute recovery data for hydrocarbons in tissue samples by the various extraction procedures is unavailable, however several of the more commonly used techniques have been compared by Farrington and Madeiros (1975) and Gritz and Shaw (1977). They found that soxhlet extraction using a benzene:methanol mixture was slightly more efficient than saponification by digestion in ethanolic potassium hydroxide. Both methods were found superior to extractions with anhydrous sodium sulfate and pentane in a blender. However, an advantage of the saponification method is that it eliminates fatty acids that can interfere with the subsequent isolation of non-polar and aromatic hydrocarbon fractions (Clark and Brown, 1977). Some authors report difficulties in the cleanup of extracts obtained by saponification of tissue samples due to the formation of intractable emulsions (Gritz and Shaw, 1977; Belisle et al., 1981). However, Belisle et al. (1981) suggested modifications to the saponification procedure (stronger alkaline solution, longer digestion at lower temperatures) which eliminate the emulsion formation.

3.4.5 Extract Cleanup and Fractionation

The organic extracts from any of the sediment or biota extraction procedures contain co-extracted lipid and non-hydrocarbon material as well as the biogenic and petroleum hydrocarbons. In order to increase the precision and discrimination in the

TABLE III-3.13

EXTRACTION METHODS FOR HYDROCARBONS IN BIOTA

EXTRACTION TECHNIQUE	SOLVENT	HYDROCARBON TYPE	REFERENCE
1. soxhlet 2. soxhlet	methanol hexane, benzene or methylene chloride	petroleum hydrocarbons	Erhardt and Heineman, 1975
soxhlet	1:1 methanol:benzene	petroleum hydrocarbons	Farrington and Medeiros, 1975
1. soxhlet 2. reflux	1:3 methanol:benzene methanolic potassium hydroxide	aliphatic and aromatic hydrocarbons	Gritz and Shaw, 1977
reflux	methanolic potassium hydroxide or sodium hydroxide, solvent partitioning	petroleum hydrocarbons	Gritz and Shaw, 1977; Farrington and Medeiros, 1975; Lee et al., 1981
1. reflux 2. reflux	hexane benzene	saturated hydrocarbons	Lawler et al., 1978
digestion	alcoholic potassium hydroxide; solvent partitioning	petroleum hydrocarbons	Warner, 1976; Dunn, 1976; Belisle et al., 1981; Smith, et al., 1984; Cretney et al., 1980
steam distillation	water	petroleum hydrocarbons (diesel oil range)	Vale et al., 1970; Ackman and Noble, 1973; Veith and Kiwus, 1977
digestion	aqueous sodium hydroxide or potassium hydroxide	total hydrocarbons	Wise et al., 1980; Albaiges and Grimalt, 1982
blending	pentane	petroleum hydrocarbons	Fong, 1976
blending	pentane plus anhydrous Na_2SO_4	petroleum hydrocarbons	Gay et al., 1980; Belisle et al., 1981; Farrington and Medeiros, 1975
1. head space sampling 2. digestion	aqueous sodium hydroxide	total hydrocarbons	Wise et al., 1980

measurement of the hydrocarbons of interest, the co-extractives are separated from the hydrocarbons prior to the quantification step. In some cases the hydrocarbons are also fractionated into one or several parts to facilitate their analysis. Cleanup and fractionation are accomplished by treatment of the extract solution by polarity, size and chemical separation techniques. Polarity separations are achieved by adsorption chromatography (column chromatography, thin layer chromatography (TLC) or high performance liquid chromatography (HPLC)) on silica, alumina or Florisil. Size separations are achieved by either gel permeation column chromatography or HPLC on Sephadex LH20, LH60 or molecular sieves. Chemical separation techniques include saponification followed by solvent partitioning, reaction with copper to remove elemental sulfur and fractionation of PAHs by partitioning into dimethylsulfoxide. The choice of separation method employed depends on both the nature of the extract solution (lipid content of sample, extraction procedure employed) and on the focus of the analysis. Several of the most commonly used cleanup and fractionation procedures are outlined in Figure III-3.4.

3.4.5.1 Polarity Separations

The purpose of polarity separations is to isolate the hydrocarbons from polar co-extracted material such as lipids. Silica or alumina columns can be used for this purpose but silica possesses the additional advantage that it also provides good resolution of saturated (non-polar) and aromatic hydrocarbons by the appropriate choice of eluting solvents. A short alumina layer on top of the silica gel column is reuired to retain high molecular weight (boiling point >350°C) polar compounds (Clark and Brown, 1977). Chromatography on partially deactivated silica used alone or in conjunction with alumina is employed to cleanup or separate hydrocarbon extracts for analysis. This technique is not standardized and a variety of chromatographic systems and solvents has been applied to the cleanup of hydrocarbon extract solutions, as outlined in Table III-3.14.

Gritz and Shaw (1977) reported higher recoveries of both saturates and aromatics from fully activated alumina/silica columns than from partially deactivated columns. However, alkene isomerization occurs more easily on activated columns (Blumer et al., 1970) and they are not recommended for the analysis of alkene-rich biological materials, especially those from unpolluted areas in which alkenes may be prominent (Gritz and Shaw, 1977). Gearing et al. (1978) compared silica column chromatographic and thin layer chromatographic separations of

Figure III-3.4 Commonly Used Hydrocarbon Extract Cleanup and Fractionation Schemes (adapted from Boehm, 1981).

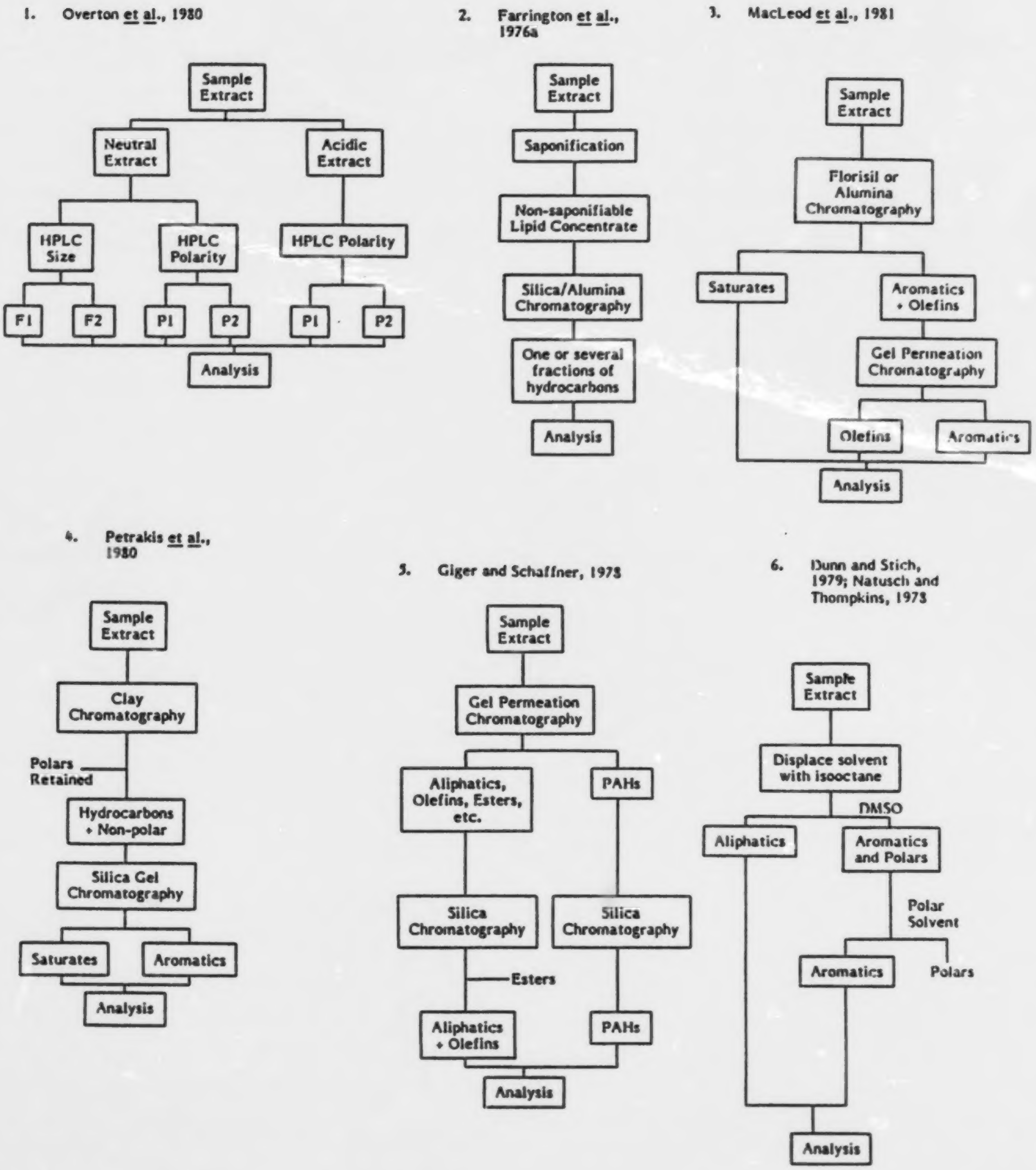

1. Overton et al., 1980
Sample Extract
Neutral Extract
Acidic Extract
HPLC Size
HPLC Polarity
HPLC Polarity
F1
F2
P1
P2
P1
P2
Analysis
2. Farrington et al., 1976a
Sample Extract
Saponification
Non-saponifiable Lipid Concentrate
Silica/Alumina Chromatography
One or several fractions of hydrocarbons
Analysis
3. MacLeod et al., 1981
Sample Extract
Florisil or Alumina Chromatography
Saturates
Aromatics + Olefins
Gel Permeation Chromatography
Olefins
Aromatics
Analysis
4. Petrakis et al., 1980
Sample Extract
Clay Chromatography
Polars Retained
Hydrocarbons + Non-polar
Silica Gel Chromatography
Saturates
Aromatics
Analysis
5. Giger and Schaffner, 1978
Sample Extract
Gel Permeation Chromatography
Aliphatics, Olefins, Esters, etc.
PAHs
Silica Chromatography
Silica Chromatography
Esters
Aliphatics + Olefins
PAHs
Analysis
6. Dunn and Stich, 1979; Natusch and Thompkins, 1978
Sample Extract
Displace solvent with isooctane
DMSO
Aliphatics
Aromatics and Polars
Polar Solvent
Aromatics
Polars
Analysis

TABLE III-3.14

EXTRACT CLEANUP AND FRACTIONATION ON SILICA OR ALUMINA/SILICA COLUMNS

CHROMATOGRAPHIC TECHNIQUE	ELUTING SOLVENTS	FRACTIONS	REFERENCE
column	1. pentane 2. 10% benzene:90% pentane; 20% benzene:80% pentane 3. benzene	saturates alkanes, 2-3 ring aromatics 4-6 ring aromatics	Farrington and Tripp, 1975, 1977; Farrington and Medeiros, 1975
column	1. hexane 2. 10% methylene chloride:90% hexane 3. 20% methylene chloride:80% hexane	- - PAHs	Albaiges and Grimalt, 1982
column	1. hexane 2. benzene	total hydrocarbons	Gritz and Shaw, 1977; Gearing et al., 1978; Hilpert et al., 1978
column	1. pentane 2. methylene chloride	- PAHs	Plumb, 1981
column	40% methylene chloride:60% pentane	PAHs	Tan, 1979
column	1. 20% benzene:80% pentane 2. pentane	total hydrocarbons	Wong and Williams, 1980
column	5% benzene:95% pentane	total hydrocarbons	Farrington et al., 1976b
column	pentane	total hydrocarbons	Farrington et al., 1976b
column	1. pentane 2. benzene	saturates aromatics	Belisle et al., 1981
column	1. petroleum ether 2. methylene chloride - petroleum ether	- petroleum hydrocarbons	Hilpert et al., 1978
column	1. hexane 2. benzene 3. methanol	saturates aromatics polars	Hilpert et al., 1978
column	1. heptane 2. benzene	saturates aromatics	Hilpert et al., 1978
thin layer plate	hexane	PAHs	Albaiges and Grimalt, 1982; Hilpert et al., 1978
thin layer plate	95% petroleum ether:5% ethyl ether - 1% acetic acid	total hydrocarbons	Farrington et al., 1976b
thin layer plate	1% ammonium hydroxide - 99% hexane	total hydrocarbons	Gearing et al., 1978
HPLC	0.5 - 10% isopropanol in iso-octane	total hydrocarbons	Hennion et al., 1983

hydrocarbons in extract solutions and found comparable results. Thin layer chromatography is more susceptible than column chromatography to photo-oxidative and evaporative losses. Recently Hennion et al. (1983) have reported a HPLC method using silica columns for the cleanup of biota extract solutions. Shorter analysis time and better reproducibility are the advantages of HPLC over column chromatography techniques.

Sample cleanup has also been accomplished by adsorption chromatography on Florisil[1] columns (Plumb, 1981; Smith et al., 1984; Belisle et al., 1981, Haddock et al., 1983; Cretney et al., 1980). No standard method is available and a variety of solvents have been employed (iso-octane, 1:1 cyclohexane:methylene chloride, 6% ethyl ether in pentane; 1:9 methylene:chloride:benzene and benzene). Florisil separations are employed for extract cleanup with no fractionation of the petroleum hydrocarbons (Belisle et al, 1981) or to fractionate the petroleum into non-polar and aromatic components (Plumb, 1981; Cretney et al., 1980).

Fractionation of hydrocarbon classes has been achieved by thin layer chromatography on cellulose acetate coated plates (Daisey and Leyko, 1979; Pierce and Katz, 1975; Plumb, 1981). Although this cleanup technique is not widely used, it is employed by the EPA in one of their two standard methods for PAHs in sediments (Plumb, 1981).

Reversed phase high performance liquid chromatography (HPLC) has been used as a polarity-based cleanup and fractionation technique (Albaiges and Grimalt, 1982; Haddock et al., 1983; Smith et al., 1984; Templeton and Chasteen, 1980; May et al., 1975; Lee et al., 1981; Hilpert et al., 1978). Octadecyl (C_{18}) or aminoalkyl (RNH_2) columns and a variety of solvent systems (methanol:water, acetonitrile: water, water:methanol:acetonitrile and isopropanol:water) have been employed. HPLC techniques are very rapid compared to column chromatography techniques and easily lend themselves to automation. As discussed above, normal phase HPLC on silica columns is used to provide hydrocarbon class separations i.e. saturates versus aromatics. Reversed phase HPLC techniques are useful for separating individual hydrocarbon components, especially aromatic compounds and are often used in finger-printing of environmental hydrocarbons or for the determination of individual compounds.

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[1] Florisil is a synthetic magnesium silicate manufactured by the Floridin company.

3.4.5.2 Size Separations

The purpose of size separations is to separate the large lipid molecules in the extract solutions from the smaller hydrocarbon molecules. Sephadex LH20 gel permeation columns are most commonly employed for this purpose (Lee et al., 1981; Plumb, 1981; Giger and Schaffner, 1978; Giger and Blumer, 1974; Warner et al., 1980) but use of Styragel and Fractosil HPLC separations have also been reported (Templeton and Chasteen, 1980). Size separations are normally employed to exclude lipid material from the extract solutions and are performed in conjunction with polarity separations used for fractionating the petroleum hydrocarbons. Giger and Blumer (1974), however, separated various PAHs from sediment extract solutions according to ring type using a combination of Sephadex LH20 gel permeation chromatography followed by a charge transfer complexation with tritrofluorenone and alumina column chromatography. This method is used as a standard EPA procedure for the analysis of PAHs in sediments (Plumb, 1981).

Use of Sepadex LH20 as the gel permeation material provides a cleanup as well as a hydrocarbon polarity separation (Giger and Schaffner, 1978). The lipid material is eluted first from the LH20 followed by the non-polar (saturated) fraction and finally the aromatic fraction. A combination of LH20 and silica chromatography have been used to effectively cleanup and fractionate sediment and biota extracts for petroleum hydrocarbon analyses (Giger and Schaffner, 1978; MacLeod et al., 1982).

Size fractionation of petroleum hydrocarbons can also be achieved using molecular sieves (commercially available with effective pore diameters of 3, 4, 5, 9 and 10Å (1Å = 10^{-10} metres; Pym et al., 1975; Dastillung and Albrecht, 1976; McTaggart and Luke, 1978). A 5 Å pore diameter will retain only straight chain hydrocarbons; a 9 Å pore diameter sieve will retain most normal and iso-paraffins and neither will retain unsubstituted aromatic hydrocarbons (Clark and Brown, 1977).

3.4.5.3 Chemical Separations

Sample extracts containing high concentrations of lipid material are often saponified prior to any fractionation steps to convert solvent-soluble wastes and glycerides to their fatty acid salts and alcohols. The polar lipids can then be partitioned into water leaving a "cleaner" extract containing non-saponifiable lipids, which includes the petroleum hydrocarbons (Boehm, 1981).

Removal of elemental sulfur from sediment extracts is required prior to GC/MS analysis of the extracts. This is accomplished by reaction of activated copper with either the entire extracts or the aromatic fraction from the silica separation (Blumer, 1957). Sulfur removal from extracts is discussed in detail in Section 3.8.5.4.

Dimethylsulfoxide (DMSO) has been used to separate PAH compounds from saturated hydrocarbons. This method utilizes coordination of the DMSO with the pi electrons of aromatic compounds and can be used to selectively partition PAHs (Natusch and Tomkins, 1978). This technique is used in a standard EPA method for the analysis of hydrocarbons in sediments (Plumb, 1981).

Aromatic hydrocarbons can also be isolated from sample extracts via charge transfer complexation reactions with trinitrofluorenone in conjunction with polarity and size separations (Giger and Blumer, 1974; Plumb, 1981).

3.4.6 Identification and Quantification of High Molecular Weight Hydrocarbons

A variety of analytical methods are available to determine the concentration and composition of hydrocarbons in the extracts of sediment and biota samples. These include:

i) non-diagnostic gravimetric measurements,

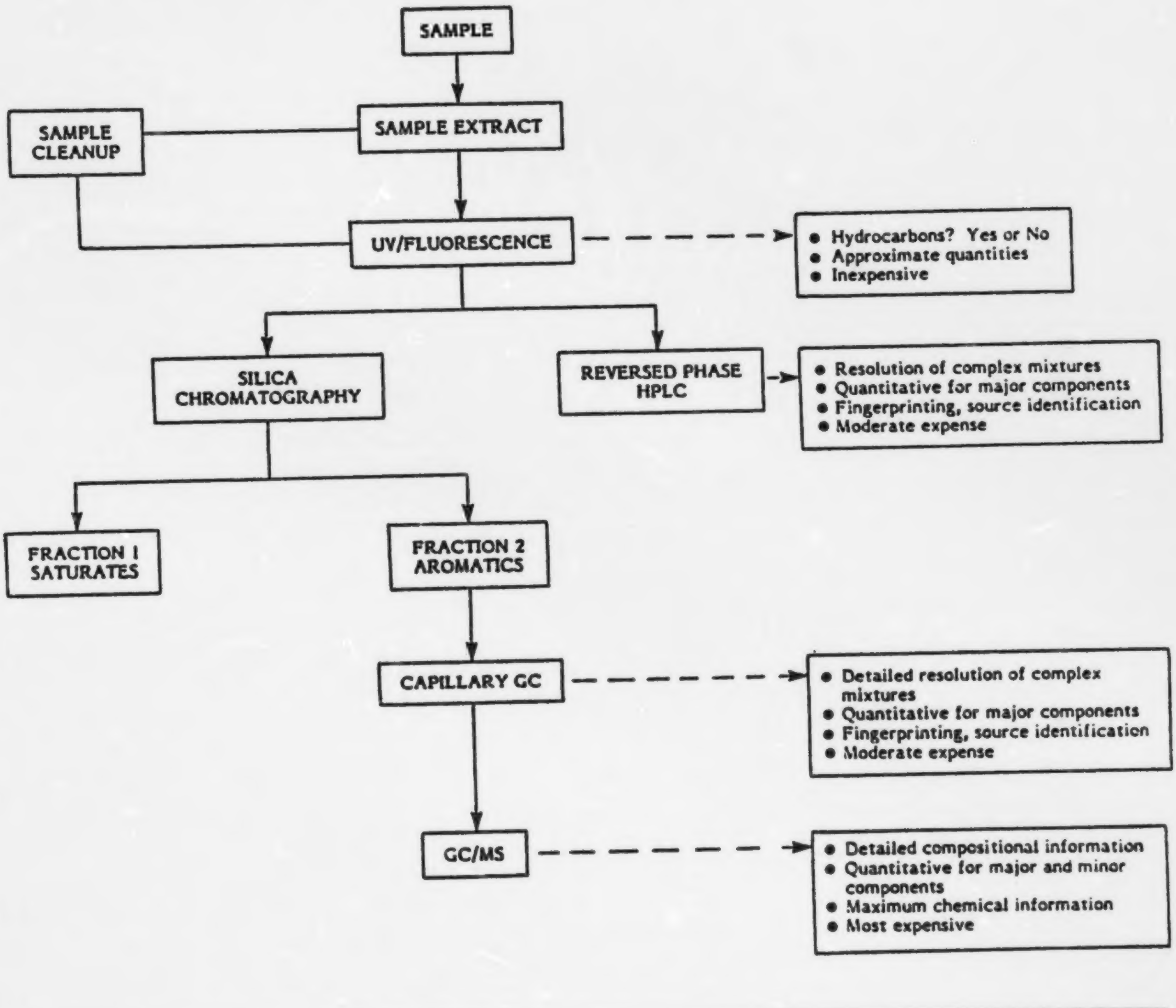
ii) gross concentration measurements by infrared spectroscopy (IR), ultraviolet absorption spectroscopy (UV) or ultraviolet fluorescence spectroscopy (UV-F) and

iii) mid to high resolution techniques of high performance liquid chromatography (HPLC), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

These techniques encompass the methods available to obtain data ranging from accurate quantification of hydrocarbons to detailed compositional profiles. As outlined in Figure III-3.5 they are often used in a hierarchial manner where screening of the hydrocarbons in the samples is followed by more rigorous procedures.

FIGURE III-3.5

HIERARCHIAL ANALYTICAL STRATEGY FOR PETROLEUM HYDROCARBONS


SAMPLE
SAMPLE EXTRACT
SAMPLE CLEANUP
UV/FLUORESCENCE
• Hydrocarbons? Yes or No
• Approximate quantities
• Inexpensive
SILICA CHROMATOGRAPHY
REVERSED PHASE HPLC
• Resolution of complex mixtures
• Quantitative for major components
• Fingerprinting, source identification
• Moderate expense
FRACTION 1 SATURATES
FRACTION 2 AROMATICS
CAPILLARY GC
• Detailed resolution of complex mixtures
• Quantitative for major components
• Fingerprinting, source identification
• Moderate expense
GC/MS
• Detailed compositional information
• Quantitative for major and minor components
• Maximum chemical information
• Most expensive

Adapted from Boehm, 1981

3.4.6.1 Gravimetry

In the gravimetric method an aliquot of the solvent is evaporated to dryness and the residue weighed on a microbalance (ASTM Method D2778-70). As little as 5 µg of material can be weighed. This method gives the total amount of non-volatile extractable material in the extract solution but does not differentiate the hydrocarbon from the non-hydrocarbon material. This result is similar to the "oil and grease" determination discussed in Section 3.5. More useful information is obtained if the gravimetric determination is carried out on the hydrocarbon fractions after the column chromatography cleanup procedures (Clark and Brown, 1977; Farrington and Tripp, 1977; MacLeod et al., 1977). The gravimetric method is subject to errors because of possible contamination of the sample by dust, because the highly volatile solvents may not dissolve all the compounds quantitatively and because the lower molecular weight hydrocarbons are sufficiently volatile that some may be lost during the solvent evaporation procedures (Clark and Brown, 1977). However, the method is rapid, sensitive and non-destructive and does not require expensive analytical equipment.

3.4.6.2 Infrared Spectroscopy

IR spectroscopy is used as a qualitative tool in the fingerprinting of petroleum hydrocarbons (ASTM Method D3414-79) for matching environmental hydrocarbons with suspected cargos of crude and fuel oils. Identification is based on comparison of the intensities of spectral bands over the entire spectrum. IR spectroscopy is also used as a quantitative tool to monitor gross concentrations of hydrocarbons in sediment extract solutions by monitoring the IR absorbance at 2920-2930 cm^{-1} (-CH_2-stretch; Boehm, 1981). The extract must first be subjected to a cleanup step to remove lipid materials (which also contain -CH_2- groups). Furthermore, measurement of the -CH_2- stretch yields measurements of only saturated molecular systems, giving no information on parent aromatics.

3.4.6.3 Ultraviolet Absorption Spectroscopy

UV spectroscopy is used to provide a rapid qualitative indication of petroleum hydrocarbons in extracted fractions, a rapid screening of extracts to identify those that contain aromatic hydocarbons and to determine conjugated polyolefins and aromatic hydrocarbons once they are isolated from other hydrocarbon material (Clark and Brown, 1977).

UV absorption spectra of the extracts are recorded over the wavelength range 210 to 350 nm. Any substance that is present in the extract and absorbs ultraviolet light at 256 nm is arbitrarily interpreted as being from petroleum sources (Clark and Brown, 1977). Due to the complicated nature of the extract, UV spectroscopy provides no information on the individual components present. However various refined products exhibit absorption maxima at different wavelengths in the ultraviolet region (these maxima have been tabulated by Clark and Brown, 1977).

UV absorption spectroscopy is a very sensitive method for the detection of aromatic compounds. However, UV absorptivity varies widely from compound to compound making calibration very difficult. Quantification can be reliable only if standards are of the same molecular composition as the unknown sample.

3.4.6.4 Ultraviolet Fluorescence Spectroscopy

UV-F (also known as spectrofluorimetry) is used to provide a rapid semi-quantitative indication of petroleum hydrocarbons in extract solutions (Boehm et al., 1982; Wakeham, 1977). UV-F is as rapid as UV absorption spectroscopy but offers advantages of greater sensitivity and selectivity for aromatic moleucles. UV-F is widely used as a screening tool because measurements can be obtained on uncleaned extracts.

UV-F methods are also used for quantification of petroleum hydrocarbons in sediment and tissue extracts (Hoffman et al., 1979; Fong, 1976). The use of UV-F as a quantitative technique is limited by the difficulty in calibrating the response. Ideally the standard should be identical in molecular composition to the petroleum in the unknown sample, but this is very difficult with environmental samples. An arbitrary reference oil may be used to calibrate the UV-F response and the results reported as "equivalent oil concentrations" (Engelhardt et al., 1984; Hoffman et al., 1979). Alternatively, chrysene solutions can be used for calibration and the results reported as "chrysene equivalents" (Cretney and Wong, 1974). Petroleum hydrocarbon determinations do not necessarily correlate with determinations by other techniques such as gas chromatography (Engelhardt et al., 1984).

Fixed wavelength UV measurements can be obtained (Cretney and Wong, 1974) but synchronous scanning UV-fluorescence is often used (Hoffman et al., 1979; Gordon et al., 1976; Fong, 1976; Boehm et al., 1982) as the latter provides some discrimination for petroleum hydrocarbons in the presence of other fluorescing material.

3.4.6.5 High Performance Liquid Chromatography

HPLC may be used in the preparative mode to cleanup and fractionate hydrocarbon extract solutions (Hennion et al., 1983) or it may be used as an analytical tool in conjunction with fixed and variable wavelength UV absorption or fluorescence detectors and refractive index detectors (Friocourt et al., 1981; Albaiges and Grimalt, 1982; Lee et al., 1981; Smith et al., 1984). Microparticulate columns can be used in the normal mode (e.g. silica columns) or in the reversed phase mode (e.g., octadecyl (C_{18}) or aminoalkyl (RNH_2) coated silica columns). A variety of solvent systems are employed: solvents such as hexane and methylene chloride for normal phase chromatography and solvents such as water, methanol and acetonitrile for reversed phase chromatography.

Hydrocarbon class separations (i.e. saturates (or non-polars) and aromatics) or resolution of individual aromatic components can be achieved by HPLC. The refractive index detector, a relatively insensitive but universal detector, is used to determine saturated hydrocarbons at high concentrations; UV absorption or UV fluorescence detectors are used to determine aromatic compounds (even at low concentrations). A calibration curve must be determined for each compound of interest because the refractive index, UV absorption and UV fluorescence vary widely from compound to compound. HPLC offers major advantages in the analysis of non-volatile and thermally labile compounds, both limitations of high resolution GC and GC/MS techniques.

3.4.6.6 Gas Chromatography and Gas Chromatography/Mass Spectrometry

Gas chromatography is widely used to analyze total hydrocarbons, classes of hydrocarbons (e.g., aromatics) or individual hydrocarbon components in extracts (Awad, 1981; Farrington et al., 1976b; Giger and Schaffner, 1978; Hilpert et al., 1978; Wise et al., 1980; Shaw, 1978; Shaw et al., 1979; Hoffman et al., 1979; Tan, 1979). Using a flame ionization detector (FID), nanogram levels of hydrocarbns can be detected. GC/MS can also be used for quantification of petroleum hydrocarbons (Cretney et al., 1980; Tan, 1979; Lao et al., 1975; Shaw, 1978; Shaw et al., 1979; Teal et al., 1978; Hites et al., 1980; Youngblood and Bloomer, 1975; Grahl-Nielsen et al., 1978; Boehm et al., 1982; Warner et al., 1980; Laflamme and Hites, 1978) but it is more difficult to calibrate especially for response to saturated hydrocarbons. The selected ion monitoring technique (SIM) is useful for determining specific components and is widely used for polycyclic aromatic hydrocarbons (PAH) (Boehm, et al., 1982, Laflamme and Hites, 1978; Shaw et al., 1979). Deuterated hydrocarbon compounds are widely used as internal standards in petroleum hydrocarbon analyses (Cretney et al., 1980; Belisle et al., 1981).

It has been found that when crude petroleum, fuel oils or extract solutions containing petroleum hydrocarbons are analyzed by gas chromatography, the chromatograms almost invariably show unresolved material beneath the resolved peaks (Boehm, 1981; Clark and Brown, 1977). This is called the envelope or unresolved complex mixture (UCM). Saxby (1978) and Butler (1975) have used the envelope shape and boiling range to obtain information on petroleum weathering processes. However, this practice may be subject to considerable error because the size of the UCM may be a function of the resolution of the particular chromatography system employed. When using gas chromatography to determine the total hydrocarbon content of extracts, it is necessary to include both the area of the resolved peaks as well as the area of the unresolved envelope.

Gas chromatography can be used to obtain a distillation curve for the petroleum hydrocarbons via the simulated distillation technique (Myers and Corry, 1984).

While the most abundant saturated hydrocarbons in extract solutions are readily identified by high resolution GC/FID techniques the minor components of most samples cannot be identified without the aid of GC/MS and acompanying computer methods (Albaiges and Albrecht, 1979). GC/MS is also valuable for the study of alkylated PAH compounds (Shaw et al., 1979; Laflamme and Hites; 1978) and nitrogen and sulfur-containing heterocyclics (Novotny et al., 1980) and for the analysis of trace amounts of aromatic hydrocarbons in extracts of sediments and biological samples containing large concentrations of interfering olefins (Warner et al., 1980; Erhardt, 1972). The use of capillary gas chromatographic columns with their high resolving capacity combined with computerized mass spectroscopy is the most powerful technique available for identifying and quantifying petroleum hydrocarbons in sediment and biota extracts (Clark and Brown, 1977; Boehm, 1981; Lao et al., 1975).

3.4.7 Extraction and Analysis of Low Molecular Weight Hydrocarbons

Hydrocarbons containing less than approximately eleven carbon atoms are not amenable to solvent extraction techniques because of their high volatility. Therefore, several specialized techniques have been developed for isolating and analyzing C_1 to C_{10} saturated and aromatic hydrocarbons in seawater, sediments and marine biota (Boehm, 1981). Methods applicable to sediment and biota samples are static headspace sampling (Friant and Suffet, 1979) and dynamic headspace purge (May et al., 1975; Bernard et al., 1978; Chesler et al., 1978; Thompson, 1979; Michael et al., 1980). Both methods rely on gas chromatography (GC/FID or GC/MS) for identification and quantification of the hydrocarbons.

In the static headspace technique a sample is placed in a closed container and heated. A subsample of the headspace gas is taken with a syringe and injected into a gas chromatograph. Rigid control of temperature and headspace volume and the use of internal standards is required for acurate quantification by this method. The dynamic headspace technique involves the continuous movement of an inert gas (e.g. helium) through the headspace of a heated flask containing the sample. The inert gas and the entrained volatile hydrocarbons are then passed through an accumulator column (e.g. Tenax GC or activated charcoal) which adsorbs the hydrocarbon material. The hydrocarbons are desorbed by heating or by extraction with a solvent such as carbon disulfide and analyzed by GC or GC/MS.

3.4.8 Summary

A variety of methods have been reported for each of the three steps (extraction, extract cleanup/fractionation and quantification) required for the analyses of petroleum hydrocarbons in sediment and biota samples. Solvent extraction is the technique most often used to isolate hydrocarbons from the sediment or biota matrix. Adsorption chromatography on dual packed alumina/silica columns is employed to clean up the extract solution and separate the hydrocarbons into saturated and aromatic fractions. Quantification is achieved by GC/FID or GC/MS.

Very few method evaluations or comparative studies of the various techniques used for the extraction of biota or sediment samples or for the cleanup and fractionation of extract solutions have been reported. However the results of several interlaboratory comparisons are available and have been summarized in Table III-3.15. These studies have been carried out on authentic environmental samples.

TABLE III-3.15

SUMMARY OF INTERLABORATORY COMPARISONS OF THE ANALYSIS OF HYDROCARBONS IN BIOTA AND SEDIMENT SAMPLES

REFERENCE	SAMPLE TYPE	HYDROCARBON TYPE DETERMINED	NO. OF PARTICIPANTS	MEAN	RELATIVE STANDARD DEVIATION	RANGE
Hilpert et al., 1978	sediment	total hydrocarbons	8	6.4 $\mu g \cdot g^{-1}$	118%	0.22 - 7.92 $\mu g \cdot g^{-1}$
			8	31 $\mu g \cdot g^{-1}$	121%	2.5 - 109 $\mu g \cdot g^{-1}$
MacLeod et al., 1982	sediment	selected individual saturates	13	49 $ng \cdot g^{-1}$ [1]	37%	27 - 84 $ng \cdot g^{-1}$
		selected individual aromatics		944 $ng \cdot g^{-1}$ [2]	65%	410 - 2600 $ng \cdot g^{-1}$
Farrington et al., 1976b	marine biota tissue	petroleum hydrocarbons (by GC)	3	740 $\mu g \cdot g^{-1}$	11%	628 - 823 $\mu g \cdot g^{-1}$
		petroleum hydrocarbons (by gravimetry)		2074 $\mu g \cdot g^{-1}$	42%	1200 - 2948 $\mu g \cdot g^{-1}$
Farrington et al., 1976b	marine biota tissue	petroleum hydrocarbons (by GC)	3	401 $\mu g \cdot g^{-1}$	29%	242 - 523 $\mu g \cdot g^{-1}$
		petroleum hydrocarbons (by gravimetry)	3	1129 $\mu g \cdot g^{-1}$	4%	1080 - 1178 $\mu g \cdot g^{-1}$
Wise et al., 1980	Alaskan mussels	saturates	8	6.2 $\mu g \cdot g^{-1}$	84%	1.5 - 13.4 $\mu g \cdot g^{-1}$
	Santa Barbara mussels	saturates	7	5.1 $\mu g \cdot g^{-1}$	75%	1.8 - 10.9 $\mu g \cdot g^{-1}$

[1] Results for C_{21}. Results for other alkanes given in original reference.

[2] Results for pyrene. Results for other aromatics given in original reference.

Therefore, the "true" hydrocarbon content and composition is not known. In addition, in all intercomparisons participating laboratories have employed their own combination of extraction, cleanup/fractionation and quantification techniques making evaluation of any single technique impossible. However, interlaboratory comparison data is useful for pointing out techniques which lead to gross analytical errors and for indicating the level of variability that can be expected in results produced by the commonly used techniques. The results of the interlaboratory comparisons reported indicate that a great deal of uncertainty is present in most results. Boehm (1981) pointed out, however, that intercalibration study results have been improving as high resolution gas chromatography or gas chromatography/mass spectrometry has been developing. With these techniques, nanogram levels of hydrocarbons in extract solutions can be determined.

The measurement of petroleum hydrocarbons in sediment and biota samples requires commonly available glassware and reagents and the use of a gas chromatograph with a flame ionization detector. To accurately determine individual saturated (or non-polar) or aromatic components, the use of capillary gas chromatography columns with flame ionization or mass spectrometric detection is recommended. The elapsed time per analysis varies between six and twenty-five hours, depending on the analysis procedure used. Soxhlet extractions require at least twenty-four hours while other types of extractions can be completed within an hour. An additonal time of approximately five hours is required to complete the analysis. A turnaround of about one to two samples per analyst per day can be expected.

Reference and intercalibration materials for petroleum hydrocarbon analysis are listed in Table III-3.16.

TABLE III-3.16

PETROLEUM HYDROCARBON REFERENCE AND INTERCALIBRATION MATERIALS

MATERIAL	TYPE	SOURCE AGENCY	CERTIFIED COMPONENTS	COMMENTS
Duramish II	Sediment	NOAA/BLM	alkanes PAH	River sediment, intercalibration material
EC-1	Sediment	CCIW	PAH	Lake sediment, certified reference in preparation
EC-2	Sediment	CCIW	PAH	Lake sediment, certified reference in preparation
	Mussel	ERNL/USEPA	26 PAH, total aromatics	Intercalibration material
SRM 1580	Shale oil	US NBS	5 PAH	Certified reference material
SRM 1647		US NBS	16 PAH	Certified concentrations in acetonitrile
SRM 1649	Urban dust	US NBS	5 PAH	Certified reference material

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3.4.10 Personal Communication

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3.5 Oil and Grease

Oil and grease is a procedurally-defined quantity which includes a range of substances with similar physical characteristics based on their common solubility in a non-polar organic solvent. "Oil and grease" is defined by the solvent used for extraction (most commonly hexane or freon). Oil and grease includes such things as hydrocarbons, fatty acids, soaps, fats, waxes, oils, elemental sulfur and any other material which can be extracted from the sample and not volatilized during the solvent removal.

The method is generally used for natural waters, domestic wastewater and industrial effluents but it is also used for sludge and sediment samples to give an indication of their total hydrocarbon content (Plumb, 1981; Inland Waters Directorate, 1979; APHA, AWWA, WPCF, 1980; Bezanson, 1981).

Oil and grease values are most useful for the measurement of petroleum-related contamination in samples in which the hydrocarbons are far more abundant than background lipophilic material (e.g., oil spills) and where no compositional information is required. The method is unable to distinguish recently spilled oil from background levels or from biogenic material. Oil and grease determinations are widely carried out because they are rapid and inexpensive. The results are somewhat dependent on the solvent chosen and are therefore often reported in terms of the solvent used (i.e., hexane extractable compounds - HEC). The detection limit of oil and grease is about 1 mg/kg. The Ocean Dumping Control Act specifies an upper limit of 10 mg/kg of oil and grease in sediments. Reported oil and grease levels in Beaufort Sea sediments are in the range 10 - 1000 mg/kg (Thomas et al., 1982).

3.5.1 Sample Handling and Storage

Sample containers and utensils are pre-cleaned by rinsing with the extraction solvent. Glass containers with teflon-lined lids are used. If samples are to be stored they are acidified to pH 2 or lower with sulfuric acid and immediately frozen at -10°C (Inland Waters Directorate, 1979). Storage time should be minimized. Sediments may lose apparent oil and grease as a result of drying and should be maintained in a field moist condition until analysis (APHA, AWWA, WPCF, 1980;

Inland Waters Directorate, 1979; Plumb, 1981). Sample size required for oil and grease analysis is typically 10 - 20 grams (wet weight).

3.5.2 Analytical Methods

Three standard methods for the determination of oil and grease in sediments or sludges have been published (Inland Waters Directorate, 1979: Plumb, 1981; APHA, AWWA, WPCF, 1980). The three methods are similar, each requiring extraction of the soluble compounds followed by a quantification procedure. Extraction is accomplished by partitioning the oil and grease into an organic solvent or by soxhlet extraction. Hexane, freon 113 (trichlorotrifluoroethane) and petroleum ether are the solvents employed in the various standard methods. Quantification is accomplished either by solvent evaporation and gravimetric determination of the residue or by monitoring the intensity of the $-CH_2-$ stretching band in the infrared spectrum of the extract solution (IR and gravimetric methods are also discussed in Section 3.4.6). Pretreatment of the sediments prior to analysis includes acidifying to pH 2 (if not already done for sample storage) and blending of the sample with magnesium sulfate monohydrate or anhydrous sodium sulfate to absorb water.

3.5.2.1 Extraction

Shaking the sediment sample with an organic solvent is the fastest and simplest method of partitioning oil and grease from the sediment sample. The disadvantages of the procedure are the possible formation of intractable emulsions during the shaking, the necessity to repeat the procedure to obtain complete extraction and the incomplete recovery of polar, heavy petroleum fractions from the sediment (APHA, AWWA, WPCF, 1980).

Soxhlet extraction is more commonly used to separate oil and grease from sediment samples. To obtain reproducible results the rate and time of extraction must be carefully controlled because of varying solubilities of the different oil and grease compounds in the extraction solvent. Polar, heavy petroleum fractions are more completely extracted by the soxhlet procedure than by solvent partitioning. However soxhlet extractions are more time consuming (four hours compared to ten minutes for partitioning by shaking).

3.5.2.2 Quantification

Two methods are used for the quantification of the oil and grease in extract solutions: gravimetry and infrared spectroscopy. Gravimetry is rapid and requires no special equipment or calibration. It does however require removal of the solvent from the extract solution. Despite the use of volatile solvents in the extraction procedure, volatile hydrocarbons may be lost during the solvent removal. This method is also subject to positive interference from elemental sulfur co-extracted from the sediment.

Infrared determination of oil and grease is the preferrred method of quantification because it is generally more precise, particularily at low oil and grease concentrations. It is the method of choice for samples that might contain volatile hydrocarbons as solvent removal is not required. However, calibration of the infrared spectrophotometer response at 2930 cm^{-1} is necessary. Standard solutions of oil and grease must be analyzed for comparison to the unknown sample. Ideally, "known oil" reference standards are used. A known oil is defined as a sample of oil and/or grease that is similar in composition to the material in the unknown sample (APHA, AWWA, WPCF, 1980). In practice "unknown oil" standards are used. These are mixtures of n-hexadecane, iso-octane and benzene at known concentrations in the extract solvent (Plumb, 1981). Multipoint calibration of the spectrometer is necessary but when amortized over many samples, it adds only a small amount of time to each analysis. No correlation of results by the infrared and gravimetric methods is available, but the infrared method could produce a positive bias for saturated non-petroleum compounds (rich in $-CH_2-$ groups) and a negative bias for aromatic compounds ($-CH_2-$ group deficient).

3.5.2.3 Other Methods

Bezanson and MacLaren Plansearch (1981) used a spectrofluorimetric and an ultraviolet spectroscopic method for the quantification of oil and grease in bottom sediments from Cape Breton. For the spectrofluorimetric method they used two standards: chrysene and Kurdistan oil, and found that the Kurdistan standards gave four times higher results than the chrysene standards at the wavelengths chosen for analysis. Chrysene standards were used for the ultraviolet method. They reported poor agreement between the results from the two methods but could not eliminate sub-sampling as a possible cause. No relationship of their results to those of traditional oil and grease methods was given, but both spectrofluorimetry and ultraviolet spectroscopy are highly specific for aromatic compounds. These methods then may have potential for estimating the petroleum portion of oil and grease, but suffer from standardization difficulties as both fluorescence and ultraviolet absorption vary widely from compound to compound.

3.5.3 Summary

The most widely used method for oil and grease in sediments is soxhlet extraction with hexane followed by solvent evaporation and gravimetric determination of the extracted material. All hexane extractable materials, including petroleum hydrocarbons and lipids, are determined by this method. Low molecular weight hydrocarbons (those which volatilize at $\leq$ 103°C) may be lost during the solvent evaporation step while co-extracted sulfur will cause a positive interference (EPS, 1979). The results give an indication of the hydrocarbon content of sediments and are most useful for monitoring high levels of hydrocarbon contamination.

3.5.4 Literature Cited

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3.6 Phenols

The terms phenols, phenolics or total phenols refer to a range of compounds with a hydroxyl functional group on an aromatic nucleus (Table III-3.17). The aromatic nucleus may contain from one to three condensed aromatic rings. Possible substituents on the phenolic molecule include hydroxyl, carboxyl, methoxyl, phenoxyl, nitro, amino, nitroso, halogen (See Section 3.7), sulfonic acid and alkyl groups. Degradation products from herbicides such as 2,4-D and 2,4,5-T are also phenolic in character.

Phenolic compounds may be toxic to aquatic organisms at mgL^{-1} levels (Bulkema et al., 1979). At μgL^{-1} levels the unpleasant taste and odour of certain phenols are apparent in drinking water and will taint edible species. Threshold values for water quality have been set by the U.S. Environmental Protection Agency and the World Health Organization (Buikema et al., 1979). For phenol itself, limits are 0.05 μgL^{-1} (EPA, 1977a) and 1 μgL^{-1} (WHO, in: Stofen, 1973). Other values range from 0.01 μgL^{-1} for 2-chlorophenol, for prevention of tainting, to 500 μgL^{-1} for 3-nitrophenol for protection of aquatic life (EPA, 1977b, c).

The uptake of some phenols appears to be reversible in fish if the organism is moved into clean water. Phenols can be eliminated as conjugated glucuronides or sulfates (Adamson, 1967; Adamson and Guarino, 1972; Adamson and Sieber, 1974; Kobayashi and Akitake, 1975; Kobayashi et al., 1976). Biotransformation into more toxic compounds and lipid accumulation have also been observed (Chambers et al., 1977; Grushko et al., 1975; Hattula et al., 1978).

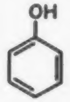
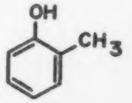
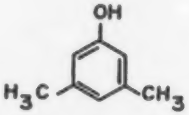
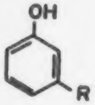
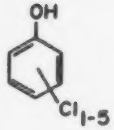
Phenolic compounds may be susceptible to microbial attack, depending on substituent type, position and number. Some phenols are subject to photochemical degradation (Buikema et al., 1979).

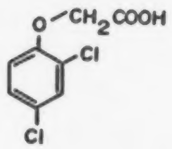
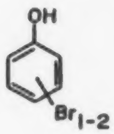
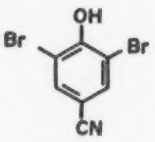
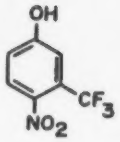
Phenols are produced synthetically for a variety of industrial uses. A minor fraction of this production is also isolated from coal tar. Phenols may enter the environment via industrial wastewaters and effluents. Industrial sources include the coking of coal, gas works, oil refineries, chemical plants, pesticide plants, wood preserving plants and dye manufacturing plants (Buikema et al., 1979). Certain phenols occur in natural resins, lignins and plant material and are therefore present as minor constituents in pulp and paper effluents. Natural phenols may also be produced in aquatic environments as algal decomposition products (Kozitskaya, 1971) as algal byproducts (Fenicel, 1975) and in marine sediments as decomposition products of humic acids and lignin of terrestrial origin (Degens et al., 1964). Hedges

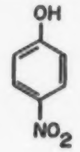
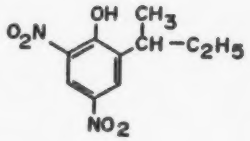
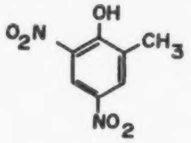
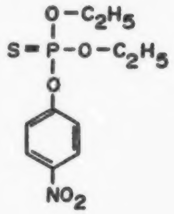
TABLE III-3.17

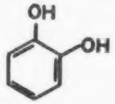
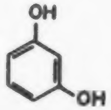
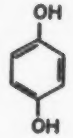
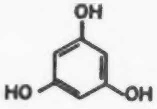
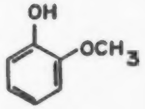
NATURAL AND INDUSTRIAL SOURCES OF SELECTED PHENOLS

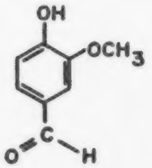
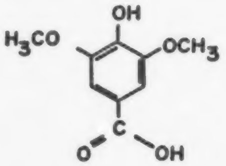
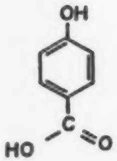
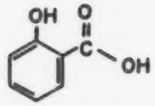
(Adapted from Buikema et al., 1979; Hedges and Parker, 1976)

COMPOUNDS	STRUCTURE	NATURAL SOURCE	SYNTHETIC/INDUSTRIAL SOURCE	INDUSTRIAL USE
Phenol		Plant material, pulp and paper mills	Coal conversion plants, coke plants, oil refineries	Phenolic resins, bisphenol A, alkylphenols
ALKYLPHENOLS				
Cresols (Methyl phenol)		Isolated from petroleum coal tar	Coal conversion plants, coke plants, oil refineries, petrochemical plants	Phenolic resins, tricresyl phosphate, disinfectant, anti-oxidants, engine-metal cleaners, ore-flotation, wire enamel solvent
Xylenols (Dimethyl phenol)			Coal conversion plants coke plants, oil refineries, petrochemical plants	
Alkylphenols			Coal gasification	Anti-oxidant (BHT), gasoline, oil, greases, plastics
HALOGENATED PHENOLS				
Chlorophenols (See Section 3.2.7)		Chlorination of phenols	Kraft pulp and paper mills, wood preserving plants, chemical plants, pesticide plants, drilling fluids	Biocides, biocide intermediates, wood preservation

COMPOUNDS	STRUCTURE	NATURAL SOURCE	SYNTHETIC/INDUSTRIAL SOURCE	INDUSTRIAL USE
2,4-D (2,4-dichlorophenoxy acetic acid) (See Section 3.2.9)			Agricultural runoff	Herbicide
Bromophenols		Excretion by red algae (Finical, 1975)		
Bromoxynil (3,5-dibromo-4-hydroxy benzo nitrile)			Agricultural runoff	Herbicide
TFM (3-trifluoromethyl-4-nitrophenol)			Agricultural runoff	Herbicide

COMPOUNDS	STRUCTURE	NATURAL SOURCE	SYNTHETIC/INDUSTRIAL SOURCE	INDUSTRIAL USE
NITROPHENOLS				
Nitrophenols			Dye plants	Dye stuff, intermediates, explosives
Dinoseb (2-sec-butyl-4,6-dinitrophenol)			Agricultural runoff	Herbicide, insecticide, acaricide, fungicide
DNOC (4,6-dinitro-o-cresol)			Agricultural runoff	Herbicide, fungicide, insecticide, acaricide
Parathion (O,O, diethyl o-(p-nitrophenyl) phosphorothioate)			Agricultural runoff	Insecticide

COMPOUNDS	STRUCTURE	NATURAL SOURCE	SYNTHETIC/INDUSTRIAL SOURCE	INDUSTRIAL USE
HYDROXYPHENOLS				
Pyrochatechol		Produced from natural resins, lignins	Chemical plants, dye plants	Antioxidants, dyes
Resorcinol			Chemical plants, dye plants	Antioxidants, eosin dyes, antiseptics, medicinals, explosives
Hydroquinone			Chemical plants, dye plants	Photographic chemicals, antioxidants, medicinals
Polyhydroxybenzenes (Pyrograllol, Gallic acid, Phloroglocinol)		Pulp and paper mills	Chemical plants, dye plants	Pigments, medicines, photographic chemnicals, dyes
METHOXY, CARBOXYL PHENOLS				
Guaiacol (Methoxyphenol)		Pulp and paper industry		

COMPOUNDS	STRUCTURE	NATURAL SOURCE	SYNTHETIC/INDUSTRIAL SOURCE	INDUSTRIAL USE
Vanillin (also vanillic acid)		Pulp and paper industry, microbial degradation of lignin and humic material		
Syringic acid		Pulp and paper industry, microbial degradation of lignin and humic material		
p-hydroxybenzoic acid (also - and m)		Pulp and paper industry, microbioal degradation of lignin and humic material		
salicylic acid (2-hydroxy benzoic acid)		Pulp and paper industry, microbial degradation of lignin and humic material		

and Parker (1976) observed that terrestrial inputs to marine sediments contain higher amounts of syringic phenols while phenols derived from marine organisms will contain higher amounts of para-hydroxyphenols.

Some phenolic compounds, of low water solubility, will concentrate in sediments compared to overlying waters. Jungclaus et al. (1978) determined levels of anthropogenic phenols in effluent, river water and sediment after discharge from a specialty chemicals plant. Sediment values ranged from $< .1$ µg·g^{-1} to 150 µg·g^{-1} for various phenols, representing an accumulation factor (relative to ambient river water) of up to five orders of magnitude for some highly lipophilic phenolss.

Data on phenols in the arctic environment are not available. As there are no industrial sources and limited decaying vegetation in the Arctic, levels can be expected to be very low.

3.6.1 Storage

Sediment samples are subject to loss of phenols by biological degradation as well as volatization. Samples should be stored wet to avoid volatilization losses during drying and thawing procedures. The Environmental Protection Agency/Corps of Engineers (Plumb, 1981) recommend storage at 4°C for not more than 24 hours. Clean glass containers, with minimum headspace, are specified, as plastic containers will absorb phenols (Afghan et al., 1974). Cleaning procedures should be the same as those used for chlorophenols (Section 3.7). $CuSO_4$ and H_3PO_4, reagents for the 4-amino antipyrine method for total phenols, may also be used as preservatives, to prevent biodegradation and precipitate interfering sulfur compounds. Biota samples or subsamples may be frozen if not analyzed immediately (Allen and Sills, 1974).

3.6.2 Pre-treatment

a) Wet sediment (10-50 g) is used directly (Plumb, 1981; Degens et al., 1964) or after sieving (Jungclaus et al., 1978) for extraction of phenols. An additional aliquot is required for dry weight calculations.

b) Biological samples, both frozen and fresh, are dried with anhydrous sodium sulfate before extraction (Hattula et al., 1978; Allen and Sills, 1974). Allen and Sills (1974) describe pre-treatment of frozen fish muscle tissue for TFM

(3-trifluoromethyl- 4-nitrophenol) analysis. The frozen fillets are chopped and then fluidized by homogenization with dry ice. CO_2 is allowed to escape (24 hours) and the sample (25 g) is mixed with anhydrous Na_2SO_4. After one hour at room temperature, the sample is extracted.

Hattula et al., (1974) describe pre-treatment of fresh, whole fish for MCPA (4-chloro-2-methyl-phenoxyacetic acid) analysis. Goldfish and sea trout (3.5 -4.5 g) are each ground in a mortar with anhydrous sodium sulfate and allowed to dry at room temperature for 24 hours before extraction.

There is little information available in the literature to determine whether or not depuration of biota is required prior to analysis. If the entire specimen is to be analyzed, depuration may be carried out to ensure that the gut contents do not bias the tissue analysis results. The appropriate depuration time is not well defined in the literature and care must be exercised to ensure that contaminants are not also being lost from the tissues. Depuration should be started as soon after sample collection as possible.

3.6.3 Extraction

3.6.3.1 Sediments

Phenols may be extracted from sediments by steam distillation or solvent extraction.

Steam distillation is the standard method for 'total phenols' in both water and sediment samples (Plumb, 1981; APHA, AWWA, WPCF, 1980; EPS, 1979; Inland Waters Directorate, 1979). Distilled water is added to the sediment and an equal volume of water is distilled over. The effectiveness of this procedure will depend on the volatility of individual compounds. Farino et al., (1981) have shown that less than 50% of several selected phenols (resorcinol, pyrogallol, m-nitrophenol, picric acid, 5-amino-1-napthol·HCl) are extracted from water by steam distillation (See also Friestad et al., 1969).

A loss of phenols during distillation has been reported to be a potential source of error (Afghan, 1974).

Three solvent extraction procedures have been utilized.

1) Degens et al. (1964) made up a 3:1 slurry of marine sediment and distilled water, acidified it to pH 1-2, saturated with NaCl and extracted 3 times with ethyl acetate. This procedure isolated natural phenolic acids, para-hydroxy benzoic, syringic, vanillic, salicylic, meta-hydroxy benzoic and para-hydroxy phenylacetic acids. Separation and identification was made by paper chromatography. No data is available on recoveries by this procedure.

2) Jungclaus et al. (1978) isolated a spectrum of industrial chemicals by soxhlet extraction of wet riverine sediment with isopropanol. The extract was then fractionated by silica gel column chromatography. HPLC, GC and GC/MS were used to separate and quantify individual compounds (phenol, cresol, alkyl, carboxylic acid and methoxy-phenols) to the 0.1 $\mu g \cdot g^{-1}$ level. No data is available on recoveries by this procedure.

3) Games and Hites (1977) also used soxhlet-extraction of wet, freshwater sediments, with 60:40 benzene:methanol. Cleanup was accomplished with silica gel column chromatography and quantification by GC/MS. The effectiveness of this procedure cannot be assessed as phenols identified in effluent and river water samples were not present in sediments at the 0.1 $ng \cdot g^{-1}$ detection limit of the method.

3.6.3.2 Biota

Methods have been developed for specific phenolic compounds in biota (see also Chlorophenols Section 3.7). In the examples described below, tissue dried with anhydrous Na_2SO_4 is solvent extracted. After appropriate cleanup procedures to remove fats, a biological extract will be essentially the same as a sediment extract and can be quantified by similar means.

Allen and Sills (1974) describe a typical procedure, analyzing TFM (3-trifluoromethyl-4-nitrophenol) in fish muscle. The tissue is dried with anhydrous Na_2SO_4 and extracted with 70:30 hexane:ethyl ether using column elution. This extract was cleaned up by back extraction into basic solution and subsequent acid partition into hexane:ethylether. This avoided possible evaporative losses on silica or resin cleanup columns. TFM was then methylated with diazomethane and determined by GC/EC. The detection limit of this procedure was 10 $ng \cdot g^{-1}$ and 75-100% recovery was achieved using spiked samples.

A second example deals with MCPA (4-chloro-2-phenoxyacetic acid) and cresols (specifically 4-chloro-o-cresol) in whole sea trout and gold fish (Hattula et al., 1978). The fish tissue was dried with anhydrous Na_2SO_4 and extracted by shaking with acidic (HCl) chloroform:diethylether. Preparative thin-layer chromatography on silica gel was used to separate the analytes from fats and fat-soluble compounds. MCPA was visualized by bromophenol spray and was recovered by ultrasonic extraction with acidic chloroform:ether (82.8% recovery). Cresols were visualized with 2-6-dichloroquinonechlorimide and recovered by diethylether extraction (83.7% recovery for 4-chloro-o-cresol). Quantification was carried out with glass capillary GC using an FID detector. Peak heights were compared with pure standards. The detection limit with this procedure is approximately 10 $ng \cdot g^{-1}$. Overall recoveries were not quoted.

3.6.4 Quantification

There are two approaches to the determination of environmental phenols: the application of procedurally-defined methods for 'total phenols' and the determination of individual compounds. 'Total phenols' methods are spectrophotometric and have been adapted for sediments from methods for water (Plumb, 1981) while methods for individual molecules utilize HPLC, GC and GC/MS (See also: Chlorophenols Section 3.7).

3.6.4.1 Total Phenols

Total phenols are determined by UV/visible spectrophotometry or spectrofluorimetry. A summary of estimated detection limits for sediments is given in Table III-3.19. Individual methods are discussed below.

a) Visible Spectrophotometry

The standard method for 'total phenols' involves the formation of an antipyrine dye, with spectrophotometric detection. The reaction is given in Figure III-3.6. This method appears in several standard manuals for water analysis (Plumb, 1981; APHA, AWWA, WPCF, 1980; EPS, 1979; Inland Waters Directorate, 1979; ASTM Part 31, 1976). The same method has been adopted for sediments by the EPA/Corps of Engineers (Plumb, 1981).

Phenols are distilled from the sediment sample as described in Section 3.6.3, to remove interferences from turbidity, colour and metal ions (EPS, 1979). They are then reacted in aqueous solution with 4-aminoantipyrine in the presence of the alkaline oxidizing agent potassium ferricyanide $K_3Fe(CN)_6$. The absorbance of the resultant dye is measured at 510 nm (Plumb, 1981). The minimum amount detectable is approximately 10 μg phenol in 500 mL of distillate (APHA, AWWA, WPCF, 1980). Assuming a 25 g sediment sample, this corresponds to a sediment concentration of 400 $ng{\cdot}g^{-1}$. For samples with lower concentrations, the distillate can be extracted with $CHCl_3$ and the absorbance measured at 460 nm (Plumb, 1981). The minimum amount detectable in a 500 mL distillate is approximately 0.5 μg phenol (APHA, AWWA, WPCF, 1980), corresponding to a sediment concentration of 20 $ng{\cdot}g^{-1}$. Sample absorbances are compared to a phenol standard curve. The method is not difficult to perform and is relatively inexpensive.

There are several limitations, however, due to non-reactivity of some compounds and various interferences. Substitution in the para-position will block the reaction, except for carboxyl, halogen, hydroxy, methoxy and sulfoxyl groups. For nitro-phenols, meta substitution also inhibits the reaction while ortho substitution blocks it (Box, 1983). The molar absorptivity of the dye complex at a given wavelength is a maximum for phenol itself and may be less for substituted phenols. The final result, in phenol equivalents, therefore represents a minimum value. A comparison between the GC and antipyrine method for phenol and cresols is given in Table III-3.18. Farino et al. (1981) give additional recoveries for a spectrum of phenols.

Steam distillable compounds such as formaldehyde will react with the reagent, causing a positive interference. Oxidizing agents such as Cl_2 in the sample, will oxidize phenols and interfere with the reaction. These can be removed by the

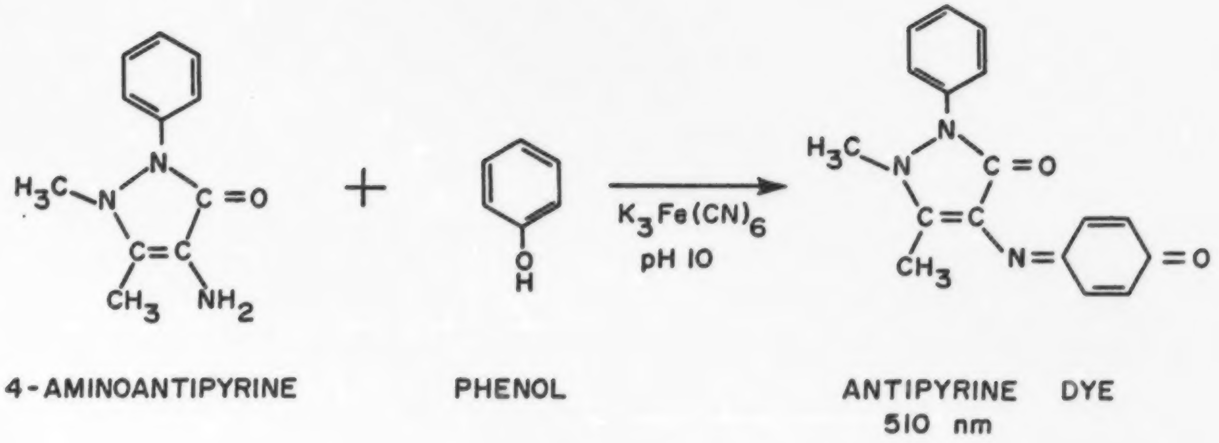

H_3C
N
C =O
C=C
CH_3
NH_2
4-AMINOANTIPYRINE
+
O
H
PHENOL
$K_3Fe(CN)_6$
pH 10
N=
=O
ANTIPYRINE DYE
510 nm

Figure III-3.6 Reaction of 4-aminoantipyrine with phenol to form coloured antipyrine dye.

TABLE III-3.18

COMPARISON OF 4-AMINOANTIPYRINE METHOD AND DIRECT GC METHOD FOR PHENOLICS IN AQUEOUS SOLUTION
(APHA, AWWA, WPCF, 1980)

	RECOVERY*	
Compound	4-aminoantipyrine	GC
Phenol	91.5 %*	91.5 %
o-Cresol	61.5 %	99.0 %
m-Cresol	37.2 %	101 %
p-Cresol	0 %	100 %

* % of weight of compound added to solution.

addition of $FeSO_4$ or $NaAsO_2$ (APHA, AWWA, WPCF, 1980). Sulfur compounds such as H_2S or SO_2 also interfere with the colour-forming reaction. Aqueous solutions can be acidified with H_3PO_4 to liberate H_2S and SO_2 at $pH < 4$. The solution is then aerated to remove the gases (APHA, AWWA, WPCF, 1980). Oils and tars can be removed from the aqueous phase by an extraction with $CHCl_3$ at pH 12-12.5. At this pH the phenolate anions remain in the water phase. Excess $CHCl_3$ is removed by careful warming on a hot water bath.

A second colorimetric technique for 'total phenols' involves coupling of phenol with 3-methyl-2-benzothiazolinone hydrazone·HCl (MBTH). The complex is oxidized with ceric ammonium sulfate ($Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$) and the absorbance is measured at 490 nm (Figure III-3.7). This method has also been accepted as a standard for water (EPS, 1979; Plumb, 1981) and has been adapted for sediments by the Environmental Protection Agency/Corps of Engineers (Plumb, 1981).

Friestad et al. (1969) compared the method with the 4-aminoantipyrine method for a range of phenols, finding that the reagent is more sensitive to para-substituted phenols and gives higher molar absortivities in general. The limit of detection in the aqueous phase is 100 $ng \cdot g^{-1}$ (for phenol) corresponding to a sediment concentration of 2 $\mu g \cdot g^{-1}$ (500 mL distillate, 25 g sediment). Interfering compounds include aliphatic aldehydes (which give high molar absorptivities), acetaldehyde, and acetone (which will block the coupling reaction).

b) Ultraviolet (UV) Spectrophotometry

Direct and differential UV spectrophotometry have also been applied to the measurement of phenols in aqueous solutions. These methods could also be used to measure phenols in an aqueous distillate from sediments, or in an organic extract, back-extracted into basic solution.

UV methods do not require derivatization of phenols and are able to measure para-blocked phenols not measured by 4-aminoantipyrine. The main disadvantage is the low molar absortivity of most phenol-dye complexes, as compared to the phenol complex itself. An additional extraction step, using organic solvents, could be used to concentrate phenols into an organic phase with a subsequent back-extraction into basic aqueous solution (Afghan, et al., 1974; Disinger and Monahan, 1982).

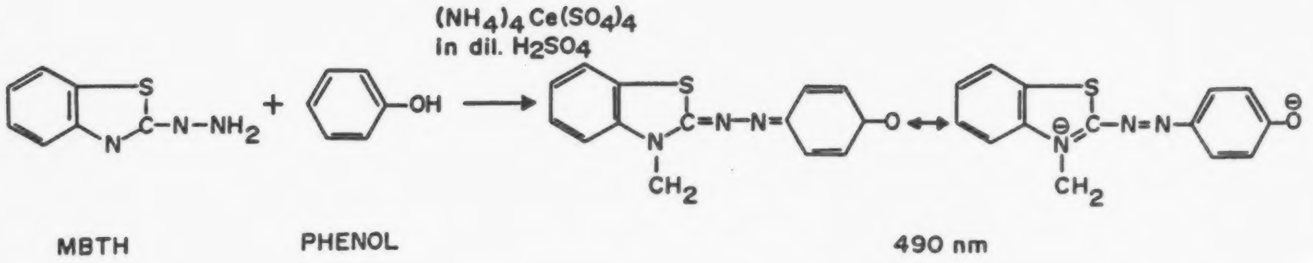

(NH4)4 Ce(SO4)4
in dil. H2SO4
S
N
C–N–NH2
+
OH
MBTH
PHENOL
CH2
O
490 nm

Figure III-3.7 Reaction of MBTH with phenol

TABLE III-3.19

ESTIMATED DETECTION LIMITS FOR TOTAL PHENOLS IN SEDIMENTS

SPECTRO-PHOTOMETRIC METHOD	CONCEN. STEP	DETECTION * LIMIT	REFERENCE
Visible: 4-aminoantipyrine	No	0.4 $\mu g \cdot g^{-1}$	APHA, AWWA, WPCF (1980)
Visible: 4-aminoantipyrine	Yes **	0.02 $\mu g \cdot g^{-1}$	APHA, AWWA, WPCF (1980)
Visible: MBTH	No	2 $\mu g \cdot g^{-1}$	Friestad *et* al.(1969)
Ultraviolet: Direct	Yes	0.1 $\mu g \cdot g^{-1}$	Afghan *et* *al*. (1974)
Ultraviolet: Differential	No	1 $\mu g \cdot g^{-1}$	Disinger and Monahan (1982)
Fluorescence	Yes	0.002 $\mu g \cdot g^{-1}$	Afghan *et* *al*. (1974)

* Estimated detection limit assuming 500 mL distillate and 10 g sediment sample.

** Note that concentration step results in a 20 fold decrease in detection limit.

Afghan et al. (1974) applied the concentration step and measured UV absorbance directly at 392 nm for phenols, the three cresol isomers and selected chlorophenols. The detection limit with this method was given as 5 $ng \cdot g^{-1}$. For a 500 mL distillate from a 25 g sediment sample, this corresponds to a concentration of 100 $ng \cdot g^{-1}$ in sediment.

The differential UV method exploits the difference in absorbance at 291 nm between unionized phenols at pH 7 and phenolate anions at pH 12 (Disinger and Monahan, 1982). The detection limit with this method is given as 50 $ng \cdot g^{-1}$ in water, corresponding to a sediment concentration of 1 $\mu g \cdot g^{-1}$ (500 mL distillate, 25 g sediment).

While this method is rapid and inexpensive, errors can arise from phenols which absorb in the unionized form and from other compounds sensitive to the pH change (amino acids, diamines, imidazole, etc.; Disinger and Monahan, 1982).

c) Spectrofluorimetry

Spectrofluorimetric methods are also able to measure para-blocked phenols without derivatization (Afghan et al., 1974; Naley, 1983). Fluorescence emission at 305 nm is measured for an acidified aqueous sample excited at 275 nm. Afghan et al. (1974) utilizing a concentration step, reported a detection limit of 0.1 $ng \cdot g^{-1}$ in aqueous solution, corresponding to a sediment concentration of 2 $ng \cdot g^{-1}$ (500 mL distillate, 25 g sediment). The method does not detect chlorophenols.

3.6.4.2 Individual Phenols

Individual phenols from various sources have been satisfactorily separated and quantified by GC, GC/MS and HPLC. Examples are given in Table III-3.20. Only two method references for phenols in sediments were found by a computerized literature search of CISTI abstracts (Jungclaus et al., 1978; Games and Hites, 1977). Derivatization procedures, which increase method specificity and sensitivity, and instrumental quantification could equally well be applied to aqueous (steam distillation) or organic solvent extracts of sediments, after appropriate cleanup.

TABLE III-3.20

SUMMARY OF HPLC AND GC METHODS FOR PHENOLIC COMPOUNDS

Reference	Analytes	Sample History	Method of Detection	Detection Limits
GAS CHROMATOGRAPHY (GC)				
McKague, 1981	Guaiacol, vanillin, 4-hydroxy-3- methoxyacetophenone	Pulp and paper effluent, XAD-2 extraction	Heptafluorobutyrate derivatives GC/EC	1 ng·g^{-1} in water (20 ng·g^{-1} in sediment)*
Coutts et al., 1981	Aminophenols	Aqueous solution, CH$_2$Cl$_2$ extract of acetate derivatives	Acetate and trifluoroacetate derivatives GC/EC GC/MS	94 mg·g^{-1} in water (2 ng·g^{-1} in sediment)
Coutts et al., 1980	σ, p-Nitrophenols, m-cresol, 1-naphthol	Environmental water samples, CH$_2$Cl$_2$ extract of acetate derivatives	Acetate derivatives GC/FID GC/MS	1 ng·g^{-1} in water (20 ng·g^{-1} in sediment)
Coutts et al., 1979	Phenol, cresols (3), 2,4-dichlorophenol, 1-naphthol	Environmental water samples, CH$_2$Cl$_2$ extract of acetate derivatives	Acetate derivatives GC/FID	7.5 ng·g^{-1} in water (150 ng·g^{-1} in sediments)
Voznakova & Popl, 1979	Phenol, σ, m-cresol, 2,6-xylenol	Environmental water samples macroreticular resin	GC/FID	1 ng·g^{-1} in water (20 ng·g^{-1} in sediments)
Jungclaus et al., 1978	Phenol, cresols, alkyl-, carboxyl-, methoxyl-phenols	Isopropanol extract of sediments	GC/MS	40 ng·g^{-1} in sediment
Games & Hites, 1977	Selected phenols, not detectable in sediments	60:40 Benzene:Methanol extract of sediments	Selected Ion Monitoring GC/MS	0.08 ng·g^{-1} in sediment
Hattula et al., 1978	MCPA (4-chloro-2-phenoxyacetic acid)	Whole goldfish, sea tract (acidic CHCl$_3$; diethylether extract derivatized with diazomethane)	Methyl derivative GC/FID	4 ng·g^{-1} in tissue
Allen and Sills, 1974	TFM (3-trifluoromethyl-4-nitrophenol)	Fish muscle tissue (Hexane: ethylether extract)	GC/EC	4 ng·g^{-1} in tissue

* Estimated detection limit assuming 500 ml distillate of 25 g sediment sample

TABLE III-3.20 cont'd.

SUMMARY OF HPLC AND GC METHODS FOR PHENOLIC COMPOUNDS

Reference	Analytes	Sample History	Method of Detection	Detection Limits
REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)				
Shoup & Mayer, 1982	Phenol, cresols, PCP	Environmental water samples, direct injection and sample enrichment by off-line hydrophobic columns and on-line switching valve and sampling pump	Electrochemical detection at carbon electrodes. LCEC	50 pg on column 1 $ng \cdot g^{-1}$ in water (Direct Injection) (20 $ng \cdot g^{-1}$ in sediment)
Kuwata et al., 1981, 1980	Phenol, cresols, ethyl phenols, xylenols, chlorophenols	Water soluble phenols (from air), derivatization with p-nitrobenzenediazonium tetrafluoroborate (NBDATFB)	p-nitrobenzeneazo (NBA) derivative. UV absorbance at 365 nm, * 254 nm	1.25 $ng \cdot g^{-1}$ in water (25 $ng \cdot g^{-1}$ in sediment)
Ogan & Katz, 1981	Phenol, cresols, xylenols, alkylphenols	Organic extracts (from coal liquefaction)	UV Fluorescence, excitation at 274 nm, emission at 298 nm.	25 pg on column
Needham et al., 1980	Phenol, 1-chlorophenol	Human serum, benzene extract	UV absorbance at 280 nm	
Sparacino & Minick, 1980	Phenol, cresols (3), xylenols (5)	Aqueous solution (from coal gasifier condensates)	UV absorbance at 215 nm to measure cresols, xylenols as group	$\mu g \cdot g^{-1}$ levels
Schabron et al., 1979	Phenol, cresols, alkylphenols, phenyl-phenols, naphthols	Organic extracts (from coal recycle solvents)	UV absorbance and fluorescence, ratios of value in basic solution to value in neutral solution	$\mu g \cdot g^{-1}$ levels
Sternson & Dewitte, 1977	o, p-aminophenol	Aqueous sample	Electrochemical detection LCEC	1 $ng \cdot g^{-1}$ in water (20 $ng \cdot g^{-1}$ in sediments)

* Estimated detection limit assuming 500 nl distillate of 25 g sediment sample

3.6.5 Standard Materials

'Total phenols' methods utilize standard curves of pure compounds for the quantification of environmental phenolic mixtures. Phenol itself is used most frequently although this leads to errors for phenols with lower reactivity with the colorimetric reagent or whose dye complex has a lower molar absorptivity.

'Individual compound' methods also use pure compounds for quantification purposes. These are usually available commercially, particularly in the case of industrial products and pesticides.

There are no reference materials, for either biological material or marine sediments.

3.6.6 Summary

The class of compounds known as phenols (hydroxyl-substituted aromatic nuclei) may be quantified as a total by spectrometry or as individual compounds by GC or HPLC.

Standard methods for Total Phenols are the 4-aminoantipyrine and the MBTH spectrophotometric procedures which use phenol itself as a standard. The result represents only a minimum value as all phenols do not react equally well with the reagents and do not give equal molar absorptivities. The 4-aminoantipyrine method, in particular, is ineffective for p-substituted phenols. Direct and differential UV spectrophotometric and spectrofluorimetric methods are available for aqueous samples and could be applied to steam distillates of sediments. HPLC and GC have been applied to the determination of individual phenolic compounds in both aqueous and solvent-extracted samples (Table III-3.20). These methods may be applied to steam distillates or to solvent extracts of sediments.

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3.7 Chlorophenols

3.7.1 Background

Chlorophenols are man-made compounds which have been generated in many industrial and agricultural operations. They are generated in the pulp bleaching process (Landner et al., 1977) and in the treatment of drinking water (Wegman and van den Broek, 1983) and used as wood preservatives (Renberg, 1974; Pierce et al., 1977) and as biocides in drilling fluids (Wells and Cowan, 1982). Chlorophenols can also enter aquatic systems as contaminants and degradation products of herbicides and pesticides (Virtanen and Hattula, 1982). Chlorophenols are highly toxic to fish (Pierce et al., 1977), and human illness and death have occurred from improper handling of treated lumber (Bevenue and Beckman, 1967). They are relatively soluble in water above pH 6 (Pierce et al., 1980) and fish have been found to concentrate chlorophenols by a factor of up to one thousand times that found in water (Rudling, 1970; Shafik, 1973; Zitko et al., 1974). Sediment concentrations can be a factor of ten higher than water concentrations (Pierce et al., 1977). Chlorophenol concentrations, measured in sediment and biota samples from various regions of the world, are summarized in Table III-3.21. The EPS has recently published a report on chlorophenols in the Canadian environment (EPS, 1981).

The chemical structures of some individual chlorophenols are shown in Table III-3.22. Pentachlorophenol is the most widely used chlorophenol and has been the subject of most investigations. All of the chlorophenols are relatively stable because of the chlorinated aromatic ring structure, which also accounts for their solubility in most organic solvents and in the lipid material of fish and other biota. The hydroxyl group is acidic, however, and can be deprotonated at high pH. The resultant chlorophenolate anions are soluble in aqueous and other polar solutions. This property of the chlorophenols is used in designing analytical methods for their determination and also may account for the accumulation of chlorophenols in sediments.

Analytical procedures for total phenolics (Section 3.6) determine chlorophenols but are unable to distinguish them from any other phenolic compounds.

The most widely used methods of analysis of chlorophenols in sediment and biota samples involve three independent steps: (1) extraction and extract cleanup, (2) derivatization and (3) identification/quantification. The extraction step, which isolates the chlorophenols from the sediment or biota matrix, is accomplished by extracting the sample with an organic solvent or an aqueous alkaline solution. In this

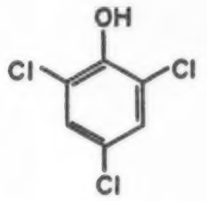
TABLE III-3.21

REPORTED LEVELS OF CHLOROPHENOLS IN SEDIMENT AND BIOTA SAMPLES

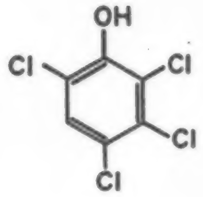
SAMPLE TYPE	LOCATION	CHLOROPHENOL CONCENTRATIONS	REFERENCE
lake sediment	Mississippi	3.0 $ng \cdot g^{-1}$ pentachlorophenol 1.0 $ng \cdot g^{-1}$ tetrachlorophenols	Pierce et al., 1980
lake sediment	Finland	0.03 - 348 $ng \cdot g^{-1}$ 2,4,6-trichlorophenol	Virtanen and Hattula, 1982
pond sediment	Mississippi	7 - 96 $ng \cdot g^{-1}$ pentachlorophenol	Pierce et al., 1977
lake sediment	Netherlands	3.6 - 30.8 $ng \cdot g^{-1}$ dichlorophenols; 0.6 - 12.6 $ng \cdot g^{-1}$ trichlorophenols; 0.9 - 4.0 $ng \cdot g^{-1}$ tetrachlorophenols; 4.6 - 8.4 $ng \cdot g^{-1}$ pentachlorophenol	Wegman and van den Broeck, 1983
lake sediment	Sweden	0.2 - 3.0 $ng \cdot g^{-1}$ pentachlorophenol	Rudling, 1970
fish (dead)	contaminated river	0.35 - 25 $\mu g \cdot g^{-1}$ pentachlorophenol	Renberg, 1974
biota	Finland lakes	0.1 - 1.1 $ng \cdot g^{-1}$ 2,4,6-trichorophenol	Virtanen and Hattula, 1982
fish	Mississippi pond	trace - 2500 $ng \cdot g^{-1}$ pentachlorophenol	Pierce et al., 1977
fish	near pulp mill	0.5 - 2.7 $\mu g \cdot g^{-1}$ 2,4,6-trichlorophenol	Landner et al., 1977
fish	Northwest Atlantic	0.5 - 11 $ng \cdot g^{-1}$ pentachlorophenol	Zitko et al., 1974
benthic biota	Germany	2.7 - 339 $ng \cdot g^{-1}$ pentachlorophenol	Ernst and Weber, 1978

TABLE III-3.22

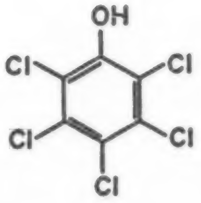
CHEMICAL STRUCTURE OF THE CHLOROPHENOLS



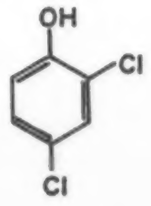
2,4,6 - trichlorophenol



2,3,4,6 - tetrachlorophenol (TCP)



pentachlorophenol (PCP)



2,4 - dichlorophenol (DCP)

step the chlorophenols are separated from the co-extractives by solvent partitioning or adsorption of chlorophenolate anions onto an anion exchange resin. A derivatization step is often desirable to convert the chlorophenols into more volatile compounds suitable for gas chromatographic analysis. The methyl ether, ethyl ether or acetate derivatives are commonly used. Identification and quantification of the chlorophenols in sediment and biota samples are carried out by gas chromatography with electron capture detection (GC/EC) or by gas chromatography/mass spectrometry (GC/MS). Using these techniques, detection limits of 0.1 $ng \cdot g^{-1}$ have been reported for chlorophenols in sediment and biota samples (Renberg, 1974; Pierce et al., 1980; Wegman and van den Broeck, 1983). A gas chromatographic analysis and a high performance liquid chromatographic analysis of un-derivatized phenols in extract solutions have also been reported.

A National Research Council of Canada publication (1982) has reviewed the methods of analysis of chlorophenols in aqueous and biological systems. The sections on cleanup procedures, derivative formation, identification and quantification are applicable to sediment and biota analyses.

3.7.2 Sample Handling

Sediment and biota samples for chlorophenol analysis must be handled carefully to ensure no loss of chlorinated phenols through volatilization or degradation. Wegman and van den Broek (1983) reported reduced recoveries of chlorophenols from spiked sediment samples stored at room temperature for two days and they recommend extraction of sediments immediately after collection. Pierce et al. (1980) however, suggest that sediments can be stored at -20°C if tightly sealed. Biota samples are wrapped in aluminum foil and immediately frozen at -20°C. Sediment samples can be collected in pre-cleaned glass containers (EPS, 1979) or in foil-lined containers (Ernst and Weber, 1978). Glassware must be washed with soap, rinsed with distilled water and heated at 325°C. Prior to use, both glassware and aluminum foil must be rinsed successively with acetone and pesticide grade hexane. For the levels of chlorinated phenols commonly encountered in sediments, samples in the range of 50 to 200 grams (wet basis) are required (Wegman and van den Broek, 1983; Pierce et al., 1977; Renberg, 1974). Approximately 5-25 g (wet weight) of tissue are required for chlorophenol analysis (Pierce et al., 1977; Renberg, 1974).

There is little information available in the literature to determine whether or not depuration of biota is required prior to analysis. If the entire specimen is to be analyzed, depuration may be carried out to ensure that the gut contents do not bias the tissue analysis results. The appropriate depuration time is not well defined in the literature and care must be exercised to ensure that contaminants are not also being lost from the tissues. Depuration should be started as soon after sample collection as possible.

3.7.3 Sample Extraction and Extract Cleanup

An extraction step is required to quantitatively isolate the chlorophenols from the sample matrix (sediment or biota). Although the extraction methods attempt to capitalize on the acidic character of the chlorophenols some neutral impurities such as PCBs, pesticides and lipids are co-extracted from the samples. These may interfere in the GC/EC quantification and are usually separated from the chlorophenols before their quantification.

3.7.3.1 Sediments

As outlined in Table III-3.23, several methods have been employed for the extraction of chlorophenols from sediment samples. Chlorophenols can be extracted (i) as neutral substances using a non-polar solvent and acidic conditions, or (ii) as neutral substances using a mixed polar-non polar solvent or (iii) as their corresponding phenolate salts using an aqueous alkaline solvent.

As noted in Table III-3.23, removal of potentially interfering co-extractives is accomplished by one of two cleanup methods. In the first, a liquid-liquid partitioning procedure, the chlorophenols are partitioned from an organic solvent into an aqueous alkaline solution to separate them from non-acidic co-extractives. The aqueous solution is acidified to reprotonate the chlorophenols which can then be partitioned into a non-polar solvent. The second method uses anion exchange resin to adsorb the deprotonated chlorophenols from an aqueous alkaline extract solution. The extract solution is discarded and the chlorophenols are desorbed from the resin by acidification followed by dissolution in an organic solvent.

These methods all result in reasonable recoveries of chlorophenols from spiked sediment samples. They differ, however, in precision of recovery and in ease of performance. Extraction by sonication is a rapid and simple technique but results in large standard deviations (38%). The more time consuming soxhlet extraction

TABLE III-3.23

EXTRACTION METHODS FOR CHLOROPHENOLS IN SEDIMENT SAMPLES

PRE-TREATMENT	EXTRACTION TECHNIQUE	CLEANUP	% RECOVERY	REFERENCE
Air-dried	Ultrasonication with benzene, acidify sediment, re-extraction with benzene	-	90 ± 38	Pierce et al., 1977
Acidify with 0.1 N HCl	20 hour reflux with 60:40 acetone:hexane	Wash with water to remove acetone, partition into 0.1 M NaOH; acidify with HCl, partition into fresh hexane	110 ± 25	Pierce et al., 1980
Air-dried	24 hour soxhlet extraction with 59:40:1 hexane:acetone:acetic acid	Wash with water to remove acetone, partition into 0.1 M NaOH, acidify with HCl, partition into fresh hexane	97 ± 9	Pierce et al., 1980
Acidify with 10^{-4} M HCl	Hand shaken with toluene	Partition into 0.1 M K_2CO_3 (3 times)	77 - 84	Wegman and van den Broek, 1983
-	Shaken with 0.2 M NaOH	Adsorption from alkaline solution onto anion exchange resin, desorption into fresh organic solvent under acidic conditions	>97	Renberg, 1974

produces high recoveries (97%) of small standard deviation (9%) but the resultant extract contains large quantities of co-extracted impurities which may interfere in the subsequent detection of chlorophenols. Although standard deviations are not reported, the sodium hydroxide extraction/anion exchange resin purification technique results in excellent recoveries (>97%), is easy to perform and requires the fewest number of sample manipulations. The use of an alkaline aqueous extraction solvent results in relatively cleaner extract solutions. Elemental sulfur and non-polar lipophilic material are not extracted. The anion exchange cleanup also avoids emulsion formation problems associated with liquid-liquid partitioning cleanup procedures (Renberg, 1981a).

Some methods call for pre-drying of the sediments. Methods requiring air-dried sediment samples did not result in lower recoveries or less precise results than methods using wet sediments. This suggests that chlorophenols are not lost in the drying step. However, no data was reported for wet and dried sediments analyzed by the same method so it is uncertain whether air-drying of sediments is actually safe or whether the recovery data in Table III-3.23 is insufficient to show its effects.

3.7.3.2 Biota

A variety of methods are used for the extraction of chlorophenols from biota samples. These are summarized in Table III-3.24. Some methods require no pre-treatment of the sample whatsoever, some require air-drying of the sample while the others acidify the tissue prior to extraction. In each case extraction is accomplished by shaking or blending the sample with an organic solvent. Hexane-isopropanol, hexane-acetone or hexane-diethyl ether are used.

Although tissue extracts are expected to contain higher levels of co-extractives than sediment extracts, their extract cleanup methods are very similar. Either the extract is partitioned with an aqueous alkaline solution to isolate the acidic chlorophenols or the chlorophenols are isolated by adsorption onto anion exchange resin. Use of the anion exchange technique can avoid the emulsion formation problems sometimes associated with liquid-liquid partitioning (Renberg, 1981a).

The methods listed in Table III-3.24 are simple to perform and require no special equipment. Only the method of Pierce et al. (1977) requires prior air-drying of the sample. Since the effect of drying on chlorophenol recoveries has not been shown, it is best to avoid it if possible. The Rudling method incorporates the

TABLE III-3.24

EXTRACTION METHODS FOR CHLOROPHENOLS IN BIOTA SAMPLES

PRE-TREATMENT	EXTRACTION TECHNIQUE	CLEANUP	% RECOVERY	REFERENCE
Homogenize in water, acidify with H_2SO_4	Hand shake with 5:1 hexane:isopropanol	Partition into borax solution, conversion to acetate and partition into fresh hexane	81 - 91[a]	Rudling, 1970
-	Homogenize with 1:2 hexane:acetone then with 2:1 hexane:diethyl ether	Shake with 1.0 M HCl, discard aqueous phase, mix with anion exchanger and 0.1 M sodium hydroxide solution, discard liquid phase, acidify, partition into benzene	74 - 92	Renberg, 1974
Air-dried	Blend with Na_2SO_4 and benzene, filter	-	62 ± 4	Pierce et al., 1977
Homogenize in water, acidify with H_2SO_4	Mix with 5:1 hexane:isopropanol	Partition into 0.1 M sodium hydroxide solution, acidify, partition into fresh hexane	44 - 104[b]	Zitko et al., 1974
Grind with Na_2SO_4, air dry	Shake with 1:1 chloroform:diethyl ether	Method of Renberg (1974)	89	Virtanen and Hattula, 1982
-	Method of Renberg (1974)	Method of Renberg (1974), conversion to ethyl ether derivitives, adsorption chromatography on silica gel		Landner et al., 1977

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[a] Includes extraction and derivatization (Section 3.7.3) step.

[b] Recovery measured for chlorobiphenylols which may be more difficult to extract than chlorophenols.

derivatization step into the cleanup procedure by conversion of the chlorophenols into their acetate derivatives before the final partitioning step. This decreases the manipulations required for the total analysis and shortens the elapsed time per analysis but imposes the limitation that only the acetate derivative can be formed. Although any of the derivatization techniques discussed below can be used in conjunction with the Virtanen and Hattula extraction and cleanup procedures, the acetate derivative constraint of the Rudling method is not serious as it is in most circumstances the derivative of choice.

3.7.4 Derivatization

Before the chlorophenols in the extract solution can be identified or quantitated by the GC/EC technique they must be converted into less polar, more volatile derivatives. The methyl ether (Pierce et al., 1977, 1980; Renberg, 1974) ethyl ether (Landner et al., 1977; Shafik, 1973; Virtanen and Hattula, 1982) acetate (Chau and Coburn, 1974; Ernst and Weber 1978; Rudling, 1970; Wegman and van den Broek, 1983; Zitko et al., 1974) and trimethylsilyl derivatives (Mattsson and Peterson, 1977) have been used for this purpose.

Factors to consider when chosing a derivatization technique include ease and completeness of derivative formation and the gas chromatographic properties (relative retention time and response) of the derivatives.

3.7.4.1 Methyl Ether Derivatives

Chlorophenols are converted to their methyl ether derivatives (chloroanisoles) by treatment of the extract (in ether) with diazomethane (CH_2N_2) for about one hour according to the procedure of Schlenk and Gellerman (1960) or by microdistillation. Diazomethane is generated by the addition of a sodium hydroxide solution to an etheral solution of N-methyl-N'-nitro-N-nitrosoguanidine. This reagent is carcinogenic and the evolved diazomethane is toxic and explosive. This method of derivatization will also derivatize any acidic pesticides, herbicides or other organic acids which may interfere with the subsequent GC/EC analysis (Chau and Coburn, 1974; Rudling, 1970). This method is simple, with very few manipulations and needs little attention.

3.7.4.2 Ethyl Ether Derivatives

Chlorophenols are converted to their corresponding ethyl ether by treatment of the extract (in ether) with diazoethane (CH_3CHN_2) according to the procedure of Stanley (1966) or by microdistillation. Diazoethane is generated from N-ethyl-N'-nitro-N-nitrosoguanidine in a reaction analogous to the generation of diazomethane. This derivatization technique is similar to the methyl ether derivatization technique but Shafik (1973) claims that the ethyl derivatives have superior gas chromatographic properties compared to the methyl derivatives.

3.7.4.3 Acetate Derivatives

Chlorophenols are converted to chlorophenol acetates by reaction of an aqueous alkaline solution of the chlorophenols with acetic anhydride. The chlorophenol acetates are subsequently partitioned into an organic solvent (hexane) leaving potentially interfering but unreacted organic acids in the aqueous phase (Rudling, 1970; Inland Waters Directorate, 1979; Chau and Coburn, 1974). This method therefore has the advantage of eliminating the herbicide interferences which can occur with the methyl or ethyl ether derivative formation. The acetate derivatization technique is less hazardous to perform and can be carried out in the alkaline solution used for partitioning the chlorophenols in the cleanup of the extract solution. The extra manipulation of acidifying the phenols and partitioning them into fresh organic solvent (as is required for methyl or ethyl ether formation) is unnecessary. An additional advantage is that the acetate derivatives of the chlorophenols exhibit a larger linear range of electron capture detector response than do the methyl ether derivatives (Chau and Coburn, 1974).

Three procedures have been reported for the formation of chlorophenol acetates.

i) Addition of an acetic anhydride/pyridine reagent to a solution of the chlorophenols in a borax buffer (Rudling, 1970).

ii) Addition of acetic anhydride to a 0.1 M sodium hydroxide solution of the chlorophenols (Chau and Coburn, 1974).

iii) Addition of acetic anhydride to a 0.1 M potassium carbonate solution of the chlorophenols (Chau and Coburn, 1974).

Acetylation procedures have been compared by Chau and Coburn (1974). They found that a sodium hydroxide solution is not very selective for the partitioning of chlorophenols from the original extract solution. The sodium hydroxide solution is so alkaline that it will dissolve even mildly acidic co-extractives. Thus, while it is a very effective medium for carrying out the acetylation reaction, it results in dirty extract solutions and many potential interferences in the GC/EC determination.

Alternatively, the borax buffer solution is mildly alkaline and selectively extracts chlorophenols from non-polar extract solutions. Unfortunately, acetylation in a borax solution requires the presence of both acetic anhydride and pyridine. The use of pyridine is undesirable for two reasons, namely the difficulty in its purification and the difficulty in its subsequent removal (small pyridine impurities give large bands in the GC/EC chromatogram). As a compromise between these two techniques, Chau and Coburn recommend acetylation with acetic anhydride in a 0.1 M potassium carbonate solution. They report 93-98% conversion of chlorophenols to their acetate derivatives by this technique with resultant solutions of similar purity to those from acetylations in borax.

3.7.4.4 Other Derivatives

Chlorophenols can also be converted to their trimethylsilyl derivatives for gas chromatographic analysis (Mattsson and Petersson, 1977; Stalling and Hogan, 1978). These derivatives are easily formed and have good GC properties but are unstable and result in large procedural blanks. They are not often used for chlorophenol quantification.

Pentafluorobenzyl (Gyllenhaal et al., 1976) and pentafluorobenzoyl (Renberg, 1981b) derivatives have also been reported.

3.7.5 Identification and Quantification

Although ultraviolet spectroscopy has been used for the detection of chlorophenols in the $\mu g \cdot g^{-1}$ range in spiked soil and sediment samples (Baker et al., 1980), this technique is not sensitive enough for the levels of chlorophenols in environmentally contaminated samples. Gas chromatography with electron capture detection (GC/EC) is the technique traditionally used to identify and quantitate chlorophenols (Chau and Coburn, 1974; Pierce et al., 1977, 1980) while gas chromatography/mass spectrometry (GC/MS) is now being used more frequently (Rudling, 1970; Landner et al., 1977; Stalling and Hogan, 1978; Pierce et al., 1980).

The chlorophenol components can be identified by GC/EC by comparison of the relative retention times (on three different GC columns) of the peaks in the chromatogram of the sample solution with the retention times of peaks due to standard chlorophenol solutions subjected to the same derivatization procedure (Chau and Coburn, 1974; Landner et al., 1977; Pierce et al., 1980; Renberg, 1974). More conveniently, chlorophenol components can be identified by GC/MS by comparison of the retention time and mass spectrum of each of the sample peaks with those of authentic compounds (Rudling, 1970; Landner et al., 1977; Pierce et al., 1977; Ernst and Weber, 1978). Quantification can be carried out by GC/EC by comparing chromatograms of sample and standard solutions. GC/MS can also be used for quantitation but calibration is more difficult (Landner et al., 1977).

Analysis of the non-derivatized chlorinated phenols on polyester-type gas chromatography columns has been reported (Edgerton et al., 1980). Diethylene glycol succinate (DEGS) and butane-1,4-diol succinate (BDS) columns were used. Pentachlorophenol could not be chromatographed on DEGS columns, while more than thirty-five minutes were required for it to elute from BDS columns. Support bonded chromatography columns were required to minimize tailing and column instability problems while scrupulous cleanup of the extract solution was required prior to the chromatography.

High performance liquid chromatography (HPLC) with ultraviolet detection has been applied to the determination of chlorophenols in (uncleaned) extract solutions (Ugland et al., 1981). Separation of nineteen chlorophenol isomers can be accomplished in thirty minutes using octadecyl (C_{18}) reverse phase columns. Ultraviolet detection is not as sensitive as the electron capture or mass spectrometer detection used with gas chromatography, resulting in higher detection limits by HPLC ($\mu g \cdot g^{-1}$ rather than $ng \cdot g^{-1}$).

3.7.6 Summary

There are no standard methods or reference materials for the analysis of chlorophenols in biota and sediment samples and no comparative evaluations have been made of the different methods. The extraction step and accompanying cleanup of the extract solution create the most variability. Choice of extraction method is based on efficiency of recovery of chlorophenols and the ease of performance of the technique. For sediments a soxhlet extraction with hexane-acetone-acetic acid or an extraction with aqueous sodium hydroxide have been successfully employed while biota are best extracted by shaking with hexane-isopropanol or chloroform-diethyl ether. Four choices of chlorophenol derivatives are available: methyl ether, ethyl ether, acetate and trimethylsilyl derivatives. The acetate derivatives possess some advantages in ease of formation and elimination of a solvent partitioning step and exhibit similar gas chromatographic properties to the methyl ether and ethyl ether derivatives. Identification and quantification of individual chlorophenols is accomplished by either gas chromatography with electron capture detection or gas chromatography/mass spectrometry.

The analysis of chlorophenols in sediments and biota requires commonly available glassware and reagents and the use of a gas chromatograph equipped with an electron capture detector. The elapsed time per analysis depends on the extraction procedure chosen. A soxhlet extraction requires twenty-four hours while most other extractions can be completed in approximately one hour. The remaining steps of the analysis require an additional time of approximately eight hours to complete. For routine analyses, samples could be analysed at a rate of about six samples per analyst per day.

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3.8 Chlorinated Hydrocarbons (Pesticides and PCBs)

Chlorinated hydrocarbons are man-made compounds of concern in the environment because of their toxicity, persistence and tendency to accumulate in biological systems (Strachan et al., 1982; Hutzinger et al., 1974). Chlorinated hydrocarbons of environmental interest include pesticides, pesticide metabolites, polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes.

Some common chlorinated hydrocarbon pesticides are given in Table III-3.25. Chlorinated hydrocarbon pesticides are also known as organochlorines, organochlorine pesticides or chlorinated hydrocarbon pesticides. A sub-classification of these pesticides is the DDT group which includes DDT and other related compounds that are based on the diphenylethane structure such as DDE, DDD and methoxychlor. Literature reports of levels of DDTs often include all chlorinated hydrocarbon pesticides derived from the DDT molecule (referred to as ΣDDT).

PCBs are chlorinated derivatives of the biphenyl molecule, shown below:

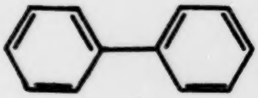


Figure III-3.8 Biphenyl Molecule

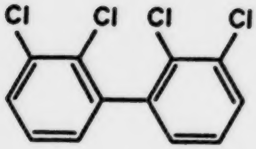
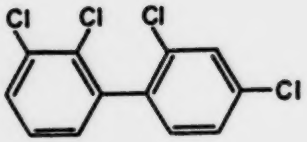
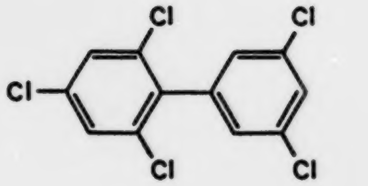
Each of the two aromatic rings can have up to five chlorine atoms so that two hundred and nine different chlorobiphenyl compounds theoretically exist (Hutzinger et al., 1974). The term PCB refers to an unspecified mixture of chlorobiphenyl compounds. Specific individual chlorobiphenyls are referred to by their systematic IUPAC names, (examples are given in Table III-3.26). PCBs have generally been manufactured and used as technical formulations or unspecified mixtures of chlorobiphenyl compounds with overall chlorine contents in the range of 20 to 80 weight percent. In North America these are the Aroclor products (from Monsanto) (e.g. Aroclor 1232, Aroclor 1260, etc). PCB levels are normally reported as equivalents of a particular technical formulation.

The primary source of pesticides in the environment are from applications for agricultural and forestry purposes and mosquito control. However, pesticides have been detected in aquatic ecosystems remote from recognizable sources (Strachan et al., 1982). Transport by ocean currents could not explain such

TABLE III-3.25 SOME COMMON CHLORINATED HYDROCARBON PESTICIDES

HCB (hexachlorobenzene)
Lindane (λ - BHC)
Chlordane
Heptachlor
Heptachlor epoxide
Endosulfan (α and β isomers)
Aldrin
Dieldrin
Endrin
DDT (o,p' and p,p' isomers)
DDD (o,p' and p,p' isomers)
DDE (o,p' and p,p' isomers)
Methoxychlor
Perthane
Kepone
Mirex

TABLE III-3.26 SOME CHLOROBIPHENYLS

Structure	Systematic Name
	2, 2', 3, 3' - tetrachlorobiphenyl
	2, 2', 3, 4' - tetrachlorobiphenyl
	2, 3', 4, 5', 6 - pentachlorobiphenyl

occurrences and aerial transport and fallout has been implicated (Bergsteinsson and Baier, 1971). PCBs have been used as plasticizers, fire retardants, paint additives and dielectric fluids (Hutzinger et al., 1974). The widespread occurrence of PCBs has also been attributed to aerial transport (Requejo et al., 1979; Eisenreich et al., 1979) and both chlorinated hydrocarbon pesticides and PCBs have been detected in marine organisms from remote areas (Weber, 1983).

Many chlorinated hydrocarbons are very stable and persistent (Lee et al., 1982; Zitko, 1980). Those that do undergo degradation tend to form compounds with persistence comparable to that of their parent compound (Strachan et al., 1982). Chlorinated hydrocarbons are lipophilic resulting in their appreciable accumulation in the lipid layers of marine organisms (Nimmo et al., 1971). Chlorinated hydrocarbons can potentially be biomagnified up the food chain (Eisenreich et al., 1980). They are only slightly soluble in sea water and possess marked affinities for particulate matter (Haque et al., 1974), resulting in higher levels in sediments than in sea water. Chlorinated hydrocarbons adsorbed onto sediments remain available to benthic organisms in the surface layers of the sediments (Fowler et al., 1978). Consequently contaminated sediments may act as reservoirs (rather than ultimate sinks) and may slowly release adsorbed chlorinated hydrocarbons, thus causing the contamination of aquatic biota for prolonged periods even after the elimination of the source (Eisenreich et al., 1979; Zitko, 1980).

Individual chlorinated hydrocarbons have been found to be quite toxic in acute or short-term testing, while a range of chronic effects on aquatic organisms has been associated with even low doses of pesticides and PCBs. These include behavioral aberrations, mutagenesis, teratogenesis or carcinogenesis from pesticides (Strachan et al., 1982) and hepatic enzyme induction, impairment of reproduction and reduced species diversity from PCBs (Roberts et al., 1978). The levels of chlorinated hydrocarbons reported for marine sediments and biota from various regions are summarized in Table III-3.27.

Analysis of sediments or marine biota for chlorinated hydrocarbons is a three step procedure involving:

1) extraction of the chlorinated hydrocarbons from the sample,

2) cleanup of the sample extract to remove interfering compounds, and

3) identification and/or quantification of the chlorinated hydrocarbons present in the extract.

TABLE III-3.27

CHLORINATED HYDROCARBON LEVELS IN SEDIMENT AND BIOTA

SAMPLE TYPE	SAMPLE LOCATION	CHLORINATED HYDROCARBONS	LEVEL*	REFERENCE
Sediment	Japan	PCBs	3 $\mu g \cdot g^{-1}$	Nakamura and Kashimoto, 1979
Sediment	Niagara River	ΣDDT PCBs	22 - 30 $ng \cdot g^{-1}$ 260 - 961 $ng \cdot g^{-1}$	Warry and Chan, 1981
Sediment	Great Lakes	chlorobenzenes	0.1 - 97 $ng \cdot g^{-1}$	Oliver and Nicol, 1982
Sediment	Lake (in Ohio)	ΣDDT PCBs	330 - 430 $ng \cdot g^{-1}$ 339 $ng \cdot g^{-1}$ (dry)	Bellar et al., 1980
Sediment	Lake Superior	PCBs	0.005 - .39 $\mu g \cdot g^{-1}$	Eisenreich et al., 1979
Sediment	Rotterdam	pesticides	0.01 - 0.9 $ng \cdot g^{-1}$	Wegman and Hofstee, 1982
Sediment	Antarctic	PCBs	13 $ng \cdot g^{-1}$	Courtney and Langston, 1981
Sediment	Beaufort Sea	PCBs ΣDDT	<5 $ng \cdot g^{-1}$ <2 $ng \cdot g^{-1}$	Thomas et al., 1982
Biota (bivalves limpets, fish)	Antarctic	PCBs	2.6 - 56 $ng \cdot g^{-1}$	Courtney and Langston, 1981
Biota (fish)	Egypt	lindane ΣDDT	35 $ng \cdot g^{-1}$ 61 $ng \cdot g^{-1}$	Saad et al., 1982
Biota (fish)	South Atlantic	ΣDDT PCBs	2 - 17 $ng \cdot g^{-1}$ 7 - 90 $ng \cdot g^{-1}$	Weber, 1983
Biota (fish)	North Atlantic	ΣDDT PCBs	0.1 - 4800 $ng \cdot g^{-1}$ 1.4 - 45,000 $ng \cdot g^{-1}$	Harvey et al., 1974
Biota (fish)	Italy	PCBs	0.1 - 6.1 $\mu g \cdot g^{-1}$	Castelli et al., 1983
Biota (benthic)	North Sea	ΣDDT PCBs	0.001 - 0.54 $\mu g \cdot g^{-1}$ 0.01 - 5.7 $\mu g \cdot g^{-1}$	Ernst et al., 1976
Biota (mussel)	Puget Sound	PCBs	46 - 487 $ng \cdot g^{-1}$	Calambokidis et al., 1979
Biota (beluga whale tissue)	Beaufort Sea	ΣDDT	2.6 $\mu g \cdot g^{-1}$	Thomas et al., 1982
Biota (ringed seal muscle)	Beaufort Sea	PCBs ΣDDT	42 $ng \cdot g^{-1}$ 27 $ng \cdot g^{-1}$	Thomas et al., 1982
Biota (arctic char)	Arctic (Northwest Passage)	PCBs ΣDDT	15 $ng \cdot g^{-1}$ 88 $ng \cdot g^{-1}$	Thomas et al., 1983

* Concentrations given on a dry weight basis for sediments and on a wet weight of tissue for biota.

Extraction of the chlorinated hydrocarbons is accomplished by a solvent extraction procedure such as soxhlet extraction or sonication. Co-extraction interferences are then removed usually by liquid - liquid partitioning or by adsorption chromatography on Florisil or alumina columns. To facilitate their quantification the chlorinated hydrocarbons are fractionated into groups usually by chromatography on Florisil or silica. The final step is the identification and quantification of the chlorinated hydrocarbons in the extract. The traditional technique used for this is gas chromatography with electron capture detection (GC/EC) although other techniques such as gas chromatography/mass spectrometry (GC/MS) are also employed.

There is no standard method for the analysis of chlorinated hydrocarbons in either sediment or biota samples. While some interlaboratory calibration studies of the various methods have been reported (Pavlou and Hom, 1976; Harvey et al., 1974; Galloway et al., 1983; Guevremont et al., 1982), most comparative work has been carried out on the individual extraction, cleanup and quantification steps of the analysis. The techniques used in each of these steps will therefore be considered separately. Using these methods most laboratories report detection limits of about 1 $ng \cdot g^{-1}$ for chlorinated hydrocarbons in biota (Ernst et al., 1976; Oliver and Bothen, 1982; Teichman et al., 1978) and in sediments (Zitko et al., 1982; Eisenreich et al., 1980; Teichman et al., 1978).

3.8.1 Sample Handling

The size of sample required for analysis is dictated by the expected level of chlorinated hydrocarbons in the sample and by the sensitivity of the analytical technique employed. With currently used analytical techniques, sediment and biota samples in the range of ten to twenty-five grams (dry weight) are adequate for the levels of chlorinated hydrocarbons commonly encountered in marine sediments and in marine biota. However the only report of chlorinated hydrocarbon levels in arctic sediments (Thomas et al., 1982) found them to be lower than normally encountered. This indicates that larger sample sizes may be required to detect the chlorinated hydrocarbons in arctic sediments and biota.

Plastic containers or utensils should not be used in the sampling, homogenization or analysis of samples as plastics have been shown to be sources of chlorinated hydrocarbon contamination (Lamar et al., 1966). Glass bottles with precleaned aluminum foil-lined lids have been reported to be the best containers for water samples although aluminum containers were also found suitable (Weil and Quentin, 1970). No similar study has been reported on containers for sediment samples but it is likely that the water sample results are directly applicable to the sediment samples. Glass or aluminum (and not plastic) containers should be used. Lids for glass bottles should be lined with teflon as foil is easily torn by the sediment. Containers and lids should be prewashed with a 1:1 hexane:acetone mixture). The sample container should be completely filled to avoid loss of chlorinated hydrocarbons to the headspace (Oliver and Nicol, 1982), and the sample put into storage immediately (see Sample Storage, Section 3.2.7.2).

Biota samples are best wrapped in aluminum foil, the air squeezed out and then placed in sealed glass or metal containers (Lee et al., 1982; FAO, 1983) and put into storage (see Sample Storage, Section 3.8.2).

There is little information available in the literature to determine whether or not depuration of biota is required prior to analysis. If the entire specimen is to be analyzed, depuration may be carried out to ensure that the gut contents do not bias the tissue analysis results. The appropriate depuration time is not well defined in the literature and care must be exercised to ensure that contaminants are not also being lost from the tissues. Depuration should be started as soon after sample collection as possible.

3.8.2 Sample Storage

Controlled sample storage conditions are required to minimize possible microbial degradation, photochemical or chemical decomposition or evaporation loss of chlorinated hydrocarbons from sediment or biota samples prior to their analysis. Storage of sediment and biota samples is discussed below.

3.8.2.1 Sediments

No studies of sediment storage conditions for chlorinated hydrocarbons have been reported in the literature. A recent EPA manual suggests that chlorinated hydrocarbons are sufficiently stable that sediment samples may be stored in field-moist, air-dried or frozen conditions (Plumb, 1981). However, the conclusions of researchers studying natural water and soil storage can be used to infer the required storage conditions for sediment samples. Substantial loss of some chlorinated hydrocarbons from soil samples stored under aerobic conditions at 25°C was observed after only seven days, while 70% loss was recorded after thirty days (Pionke et al., 1968). Decomposition of several chlorinated hydrocarbons was also reported in spiked river water samples stored for eight weeks in sealed containers at room temperature under sunlight and fluorescent light (Eichelberger and Lichtenberg, 1971). Lee et al., 1982, therefore recommended that sediment samples should be stored in an inert atmosphere (argon or nitrogen) at <4°C in the dark and extracted as soon as possible. The extracts can then be stored at -20°C for a few weeks before conducting the cleanup and quantitative steps of the analysis. In a study of sediment storage conditions currently in progress at the Canada Centre for Inland Waters, no degradation of chlorinated hydrocarbon pesticides or PCBs stored at -20°C has been detected for eight weeks (H.B. Lee, personal commun.). Addison and Nearing (1982) and Guevremont et al., (1982) recommend immediate freeze-drying of the sediment followed by storage at <4°C in sealed aluminum containers. The FAO (Food and Agricultural Organization of the United Nations), however, suggests that the freeze-drying process itself may alter the sample (FAO, 1983).

3.8.2.2 Biota

The FAO suggests that biota samples can be stored frozen for up to six months (FAO, 1983). French and Jeffries (1971) investigated the use of preservatives such as formaldehyde and phenol as an alternative to deep freeze storage of biota tissue for chlorinated hydrocarbon analysis. They reported extensive loss (89%) of DDT from tissue stored in 4% formaldehyde at room temperature for one year, similar loss of DDT from tissue stored at -14°C, but no loss of DDT from tissue stored in 10% phenol at room temperature. However, no other reports of room temperature preservative storage of biota for chlorinated hydrocarbon analysis have been found and biota are usually stored frozen (<-18°C) until analyzed (Lee et al., 1982; Oliver and Nicol, 1982; Galloway et al., 1983).

3.8.3 Sample Pretreatment

3.8.3.1 Sediments

Frozen sediment samples are thawed at room temperature immediately prior to analysis. The sediment is then prepared for extraction of the chlorinated hydrocarbons by one of the following procedures:

1) Free water is decanted from the sample and discarded. The sediment is extracted wet. (Bellar et al., 1980; Plumb, 1981; Oliver and Bothen, 1982; Inland Waters Directorate, 1979; Eisenreich et al., 1980).

2) Free water is separated from the sample by centrifugation. The sediment is extracted wet. (Goerlitz and Law, 1974).

3) Free water is separated from the sample by filtration with a glass fibre filter. The sediment is extracted wet. (Goerlitz and Law, 1974).

4) Water is removed from the sample by oven drying at 60°C. The sediment is extracted dry. (Mattsson and Nygren, 1976).

5) Water is removed from the sample by air-drying. The dry sample is ground with a mortar and pestle. Water is added (approximately 10%) and the sediment is extracted wet. (Bellar et al., 1980; EPS, 1979).

No comparison of these pre-extraction treatments of sediment samples have been reported in the literature, however, it is known that chlorinated hydrocarbons are more efficiently extracted from wet soil samples than from dry soil samples (Chiba, 1969). Although Zitko (1980) cautions against the loss of the more volatile chlorinated hydrocarbons during the drying of sediments, this is not the cause of better extraction efficiencies with wet soil samples. Increased recovery of chlorinated hydrocarbons from pre-dried soils can be achieved by the addition of twenty to forty weight percent water prior to the extraction step (Williams, 1968; Saha, 1971). Similar results can be expected from sediments and in fact good recoveries of chlorinated hydrocarbons have been reported from sediment samples with a water content ranging from 10% to 75% (Lee et al., 1982) and from air-dried moisture-added (10%) sediment samples (Bellar et al., 1980).

However, Pavlou and Hom (1976) reported the results of an interlaboratory calibration study on chlorinated hydrocarbon analyses of marine sediments and drew conclusions contradicting these results. Ten laboratories participated, each using its own pre-treatment, extraction, cleanup and quantification procedures. The authors found that the laboratories who analyzed dried or partially dried sediment reported significantly higher PCB levels than those who analyzed wet sediment. They concluded that sediments must be dried before extraction. However, if the laboratory which partially dried the sediment (by filtering off excess water) is moved from the dry category to the wet category, the PCB levels reported by the two groups are comparable (664 ± 54 $ng \cdot g^{-1}$ for dried sediments versus 538 ± 177 $ng \cdot g^{-1}$ for wet sediments). In addition, the chlorinated hydrocarbon pesticide levels (reported by only six of the ten laboratories) were significantly lower if the sediment was dried before extraction (5.62 ± 0.86 $ng \cdot g^{-1}$) than if it was analyzed wet (44.6 ± 25.1 $ng \cdot g^{-1}$). Due to the large overall variability in the results reported (25% relative standard deviation for PCBs and 88% relative standard deviation for chlorinated hydrocarbon pesticides), the small number of participants in the study, and the variability in the subsequent extraction, cleanup and quantification procedures used by each of the laboratories, the claim that sediments must be pre-dried cannot be substantiated by the data presented.

From the results of Lee et al., (1982) and Bellar et al., (1980) it is recommended that some water be present during the extraction step. If excess water is removed from the sediment by one of the methods outlined above, care should be taken not to lose any of the volatile compounds by excess heating or exposure of the sample and not to bias the sample by loss of fine-sized sediment material.

Goerlitz and Law (1974) recommended that material coarser than 2.0 mm in diameter be removed from the sediment sample prior to analysis because of the possible presence of larger benthic organisms which could embody a significant portion of the chlorinated hydrocarbons. Bellar et al., (1980) and Addison and Nearing (1982) advocate grinding of the dried sediment in a mortar and pestle to obtain a fine powder, which is then wet with water and extracted. No data is presented to indicate whether grinding of the sample is actually required.

3.8.3.2 Biota

Frozen biota samples are thawed at room temperature, unwrapped immediately prior to analysis and analyzed without loss of fluid (Oliver and Bothen, 1982; Galloway et al., 1983; FAO, 1983). Several methods prepare tissue samples for extraction by dry maceration with sand and sodium sulfate (EPS, 1979; EPA, 1980).

3.8.4 Sample Extraction

The extraction step is required to quantitatively isolate the chlorinated hydrocarbons from the natural matrix (sediment or biota). An efficient extraction method which exhaustively removes the chlorinated hydrocarbons from the sample is required. Chlorinated hydrocarbon pesticides and PCBs are extracted simultaneously from sediment and tissue samples. They can be separated by subsequent fractionation procedures if required for identification and/or quantification.

3.8.4.1 Sediments

A summary of the methods reported in the literature for the extraction of chlorinated hydrocarbons from sediment samples is given in Table III-3.28. While a variety of mechanical means (shaker, steam distillation, soxhlet extraction, column elution and sonication) and solvent systems (acetonitrile, acetone, hexane and a range of acetone-hexane mixtures) are in use, relatively little comparative research has been conducted on the efficiency of each of the methods employed.

The data presented in Table III-3.28 suggest that almost all the extraction procedures produce reasonable recoveries (>80%, Plumb (1981)) of chlorinated hydrocarbons from the sediments analyzed. However, most of the data shown was collected by analyzing samples spiked with the chlorinated hydrocarbons rather than contaminated environmentally. Quantitative recovery of a spike does not guarantee quantitative recovery of analyte in the sediment matrix. Bellar et al., (1980) found that soxhlet extraction using a hexane:acetone solvent mixture (90:10) extracted significantly more PCBs from a sample of contaminated lake sediment than did either an ultrasonic extraction with acetone or a steam distillation with water and hexane. These authors also found that the same three extraction procedures all worked equally well for uncontaminated sediment samples which had been spiked with a PCB mixture (Aroclor 1254). They therefore concluded that efficient recovery of chlorinated hydrocarbons can be achieved by most extraction techniques when analyzing spiked samples but that all extraction techniques are not equally suitable for analyzing environmentally contaminated samples. Their results suggest that soxhlet extraction with a hexane:acetone solvent is more efficient than steam distillation or sonication with acetone at extracting chlorinated hydrocarbons from sediments.

These findings are supported by the data of Wegman and Hofstee (1982). They obtained lower recoveries from samples analyzed thirty days after spiking compared to similar samples analyzed two days after spiking. Assuming that none of the spike was lost in the thirty day period, results could indicate that spiked material in the thirty day samples had interacted with the sediment and hence is more similar

TABLE III-3.28

EXTRACTION OF CHLORINATED HYDROCARBONS FROM SEDIMENT SAMPLES

METHOD		SOLVENT	% RECOVERY	SPIKED[d]	UNSPIKED[e]	COMPOUNDS AND LEVELS	REFERENCE
Soxhlet		hexane	< 95%	X		20 $ng \cdot g^{-1}$ PCBs	Flinn et al., unpubl.
Soxhlet		hexane	88-94	X		0.1 $\mu g \cdot g^{-1}$ mixture of PCBs (Aroclor 1254)	Addison and Nearing, 1982
Soxhlet	41:59	hexane:acetone	-		X	0.1 - 330 $ng \cdot g^{-1}$ mixture of chlorobenzenes	Oliver and Nicol, 1982
Soxhlet	41:59	hexane:acetone	80-98	X		2 - 24,000 $ng \cdot g^{-1}$ chlorobenzenes	Oliver and Bothen, 1982
Soxhlet	41:59	hexane:acetone	88	X		0.25 $\mu g \cdot g^{-1}$ toxaphene	Hughes et al., 1970
Soxhlet	9:1	hexane:acetone	92	X		2-10 $ng \cdot g^{-1}$ mixture of PCBs (Aroclor 1254)	EPS, 1979
Soxhlet	65:35	hexane:ether	103	X		4 $\mu g \cdot g^{-1}$ kepone, 340 $\mu g \cdot g^{-1}$ PCBs	Saleh and Lee, 1978
Hand Mixed		acetone	< 90 60-80[a]	X		1 - 5 $ng \cdot g^{-1}$ mixture of PCBs	Kerkoff et al., 1982
Shaker	2:1 4:1	hexane:acetone and hexane:acetone	75-99	X		0.5 - 1 $ng \cdot g^{-1}$ mixture of chlorinated hydrocarbon pesticides	Goerlitz and Law, 1974
Shaker	1:2	hexane:acetone	-		X	0.02 - 0.50 $ng \cdot g^{-1}$ mixture of chlorinated hydrocarbon pesticides	Mattsson and Nygren, 1976
Steam Distillation		water	80-90	X		mixture of PCBs	Eisenreich et al., 1980
Soxhlet (dry[c])		petroleum ether	27-59	X		0.1 $ng \cdot g^{-1}$ mixture of chlorinated hydrocarbon pesticides	Wegman and Hofstee, 1982
Soxhlet	1:1	acetone:petroleum ether	42-87	X			
Hand Shaken	1:1	acetone:petroleum ether	70-79	X			
Hand Shaken		acetone	76-90	X			
Hand Shaken (dry[c])		petroleum ether	47-56	X			
Hand Shaken (dry[c])	1:1	acetone:petroleum ether	75-86	X			
Soxhlet	90:10	hexane:acetone	100[b]		X	340 $ng \cdot g^{-1}$ mixture of PCBs	Bellar et al., 1980
Sonication		acetone	74		X		
Steam Distillation		water	75		X		
Soxhlet	90:10	hexane:acetone	109	X		300 $ng \cdot g^{-1}$ mixture of PCBs (Aroclor 1254)	Bellar et al., 1980
Sonication		acetone	103	X			
Steam Distillation		water	99	X			
Column	1:1	hexane:acetone	-		X	mixture of chlorinated hydrocarbon pesticides	Thompson, 1977; Plumb, 1981
Blender		acetonitrile	-			chlorinated hydrocarbon pesticides and PCBs	Inland Waters Directorate, 1979; Plumb, 1981

[a] The three most volatile PCB components gave low recoveries by this method.

[b] Recovery defined to be 100% to permit comparison with other methods.

[c] Sample was first thoroughly mixed with anhydrous sodium sulfate until a dry powder was obtained.

[d] The chlorinated hydrocarbons were incorporated into the sample in the laboratory.

[e] The sample was environmentally contaminated with chlorinated hydrocarbons.

to "real" samples. Recoveries based on spiked samples should therefore be used with caution. Such data cannot be used to estimate the actual accuracy of a particular method but can be used to identify methods which exhibit poor or variable recoveries and which should therefore not be used.

Besides efficiency of chlorinated hydrocarbon recovery, a second consideration in the choice of an extraction method is the cleanliness of the extract obtained. The more efficient the extraction method for chlorinated hydrocarbons, the greater the quantity of co-extractives from the sample matrix and the more rigorous the cleanup procedure must be. A compromise extraction method may therefore be chosen - one which gives a reasonable recovery of chlorinated hydrocarbons while producing a manageable quality of extract solution.

The Water Quality Branch, Inland Waters Directorate of Environment Canada employs ultrasonic extraction with acetone to extract chlorinated hydrocarbons from sediments (Lee et al., 1982). To ensure complete recovery they repeat the extraction twice. With this procedure they reported excellent recoveries of pesticides and PCBs and found that their extract solutions were cleaner than those from soxhlet extraction. Although Bellar et al., (1980) obtained cleaner extracts from steam distillation extractions than from either soxhlet extractions or from sonications, they reported poor recoveries (75%) of chlorinated hydrocarbons from environmentally contaminated sediments by the steam distillation procedure and it is not recommended for quantitative work. The EPA (Plumb, 1981) and the Inland Waters Directorate (1979) also extract chlorinated hydrocarbons by blending the sediment with acetonitrile. The procedure is repeated twice to ensure complete recovery of chlorinated hydrocarbons while limiting the amount of co-extracted interferences.

A comparison of the various extraction techniques is presented in Table III-3.29. The equipment and material required to perform a soxhlet, an ultrasonic extraction or a blender extraction is inexpensive and likely on hand in most laboratories. The data in the literature indicate that any of these methods will give satisfactory results for sediment analysis. The main advantage of the soxhlet technique is efficiency of recovery. The disadvantages are the large amount of co-extractives and the long time required per extraction (typically twenty-four hours but up to seventy-two hours (Oliver and Bothen, 1982)). The advantages of the sonication and blender techniques are that they are fast (about one minute per extraction) and result in cleaner extracts. The disadvantage of these methods is that the extraction must be repeated twice to ensure recovery of the chlorinated hydrocarbons, resulting in extra sample handling and therfore more possibility of experimental error.

TABLE III-3.29

ADVANTAGES AND DISADVANTAGES OF COMMONLY USED EXTRACTION TECHNIQUES

EXTRACTION TECHNIQUE	ADVANTAGES	DISADVANTAGES
Soxhlet Extraction	- most efficient extraction technique - ease of performance - uses readily available, inexpensive equipment	- large quantity of co-extractives - long extraction times required
Ultrasonic Extraction and Blender Extraction	- medium efficiency of extraction - very short extraction times - cleaner extract solutions obtained than with soxhlet extraction - uses readily available, inexpensive equipment - ease of performance	- extraction must be repeated twice to ensure recovery - extra sample handling may increase analytical uncertainly
Steam Distillation	- uses readily available, inexpensive equipment - ease of performance - produces cleanest extraction solutions	- incomplete recovery of chlorinated hydrocarbons - long extraction times required
Hand Mixing, Shaker or Rotation	- uses readily available, inexpensive equipment - ease of performance	- long extraction times required - extraction must be repeated to ensure recovery

Other extraction methods reported in Table III-3.28, such as column extraction and hand mixing have not been compared to the other more commonly used methods. The EPA (Plumb, 1981) recommends column extraction as an alternative extraction method.

3.8.4.2 Biota

Several methods are commonly employed for the extraction of chlorinated hydrocarbons from biota samples. Efficiency of chlorinated hydrocarbon extraction has not been reported for the individual methods, so choice of method can be based on convenience and on the equipment at hand. The methods are:

i) Soxhlet extraction of homogenized sample with a 1:1 chloroform-methanol mixture (Porter et al, 1970) or with methylene chloride or benzene (Galloway et al., 1983).

ii) Extraction (by blending and filtration) of homogenized sample with a polar solvent such as acetonitrile (Inland Waters Directorate, 1979; Plumb, 1981) or acetone (Galloway et al., 1983).

iii) Blending of the sample with anhydrous sodium sulfate, followed by extraction (by homogenization and filtration) with ether (Porter et al., 1970) or ether followed by acetonitrile (EPA, 1980) or hexane (Weber, 1983; Shaw and Connell, 1984) or dichloromethane (Yamato et al., 1983; Galloway et al., 1983) or a 3:1 hexane:isopropanol mixture (Galloway et al., 1983).

iv) Blending of the sample with anhydrous sodium sulfate. The sample/sodium sulfate mixture is then packed in a chromatography column and extracted with petroleum ether or cyclohexane (Hesselberg and Johnson, 1972) or 1:1 hexane:acetone (Plumb, 1981).

v) Blending of the sample with anhydrous sodium sulfate. The sample/sodium sulfate mixture is soxhlet extracted with a 41:59 hexane:acetone mixture (Oliver and Bothen, 1982) or a 9:1 hexane:acetone mixture (EPS, 1979) or petroleum ether (Teichman, et al., 1978).

vi) Extraction of sample with a 35:10 acetone:hexane mixture and then with a 9:1 mixture of hexane:diethyl ether (by mixing and filtration). (FAO, 1983).

3.8.5 Extract Cleanup

An efficient extraction method which exhaustively removes the chlorinated hydrocarbons from a sample also yields considerable amounts of co-extractives from the sample matrix. These include lipids from biological tissue, organophosphorus and carbamate pesticides from sediments and biological tissue and sulfur from sediments. These co-extractives can interfere in the gas chromatographic identification/quantification step by completely masking the presence of some of the chlorinated hydrocarbons of interest or by being mistaken for chlorinated hydrocarbons and causing falsely high levels to be reported. These interferences can be minimized by the use of a rigorous cleanup procedure to separate the interfering substances from the extract. Several steps are required for the complete cleanup of an extract prior to quantification by GC/EC. These include:

i) Separation of fats, lipids, waxes or other polar co-extractives from the extract.

ii) Fractionation of the chlorinated hydrocarbons into groups to simplify the subsequent GC/EC analysis.

iii) Removal of elemental sulfur from the extract solution (sediments only).

iv) Evaporation of excess solvent to concentrate the extract components.

The techniques used to accomplish each of these steps will be examined separately but a comparative summary of each is given in Table III-3.30.

3.8.5.1 Liquid - Liquid Partitioning

Liquid-liquid partitioning is widely used as a first cleanup step for the removal of polar co-extractives from extract solutions. The extract solution, which

TABLE III-3.30

SUMMARY OF CLEANUP STEPS EMPLOYED FOR SEDIMENT AND BIOTA EXTRACTS

CLEANUP STEP	PURPOSE	ADVANTAGES	DISADVANTAGES
liquid - liquid partitioning	- remove polar co-extractives - replace non-volatile solvents	- minimal cleanup accomplished - quickly removes non-volatile solvents without loss of chlorinated hydrocarbons	- only effective for biota
adsorption chromatography on Florisil	- remove lipid co-extractives - fractionate chlorinated hydrocarbons into groups	- simultaneously removes lipids and fractionates chlorinated hydrocarbons	- reproducible fractionation can be difficult due to variability in Florisil batches
adsorption chromatography on alumina	- remove lipid co-extractives	- very efficient removal of lipids - not very susceptible to differences in batches or degree of activation	- a subsequent silica fractionation of chlorinated hydrocarbons is required
adsorption chromatography on silica	- fractionate chlorinated hydrocarbons into groups	- separation of most chlorinated hydrocarbon pesticides from PCBs	- does not remove lipids efficiently - performance depends on degree of activation of column which is difficult to reproduce
adsorption chromatography on charcoal/polyurethane foam	- fractionate chlorinated hydrocarbons into groups	- efficient removal of lipids - efficient separation of chlorinated hydrocarbons	- adsorbent must be prepared, time consuming
gel permeation chromatography (Bio-Beads SX-2, Sephadex LH20)	- remove lipid co-extractives	- very efficient removal of lipids - very efficient recovery of chlorinated hydrocarbons - can be automated	- does not fractionate chlorinated hydrocarbons - medium handling difficulty

can be in either a polar or a non-polar solvent, is partitioned with an immiscible solvent of opposite polarity; the polar co-extractives dissolve in the polar solvent which is then discarded. Typically hexane is used as the non-polar phase and acetonitrile or water/acetone is used as the polar phase (Chau and Babjak, 1979; Oliver and Bothen, 1982; Goerlitz and Law, 1974). Often sodium chloride or sodium sulfate is mixed with the water so that the chlorinated hydrocarbons are "salted out" from the polar phase and are efficiently recovered in the non-polar phase (Plumb, 1981).

This liquid-liquid partitioning step is useful for the removal of co-extractives from biota samples but there is no significant advantage in using it for the cleanup of sediment extracts (Lee et al., 1982). It is commonly used with sediment extracts, however, to provide a means of replacing a polar solvent with a non-polar one to facilitate subsequent cleanup steps.

3.8.5.2 Adsorption Chromatography

Adsorption chromatography is widely used technique for cleanup of sample extracts prior to gas chromatographic analysis. The popularity of this technique is likely due to the fact that it can be used not only to separate fatty products from the extract solution but at the same time effect a preliminary fractionation of the chlorinated hydrocarbons if gradient elution is applied to the column (Lee et al., 1982). Silica, alumina and Florisil[1] are all employed as solid adsorbents, but Florisil is most commonly used. Silica and alumina chromatography are difficult to apply on a routine basis due to the variability of the adsorbent from batch to batch and the possible loss of chlorinated hydrocarbon components. More commonly, adsorption chromatography on Florisil is used to fractionate the pesticides and PCBs in extracts that have been cleaned by some other method (e.g. by GPC, see Section 3.8.5.3; EPA, 1980).

The chlorinated hydrocarbons can be eluted from the Florisil by petroleum ether, followed by 6% diethyl ether in petroleum ether, followed by 15% diethyl ether in petroleum ether and finally 50% diethyl ether in petroleum ether (Plumb, 1981). This yields four fractions of chlorinated hydrocarbons. The first contains the PCBs, DDE, BHC, aldrin and heptachlor, the second contains DDT, DDD, lindane, chlordane, methoxychlor and heptachlor epoxide the third contains endrin, alpha-endosulfan and dieldrin and the fourth contains beta-endosulfan.

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[1] Florisil is a synthetic magnesium silicate manufactured by the Floridin Company.

A modification to this diethyl ether/petroleum ether system was proposed by Mills et al. (1972). The eluting mixtures they employed were: (1) 20% methylene chloride in hexane, (2) 50% methylene chloride: 0.35% acetonitrile: 49.65% hexane and (3) 50% methylene chloride: 1.5% acetonitrile: 48.5% hexane. They reported improved cleanup of fats and oils while maintaining essentially complete recovery of the chlorinated hydrocarbons. While this modification would have no advantage over the diethylether/petroleum ether method for sediment extracts, it might produce superior results for lipid-rich biota extracts. However, the Mills modification has not been widely adopted.

Deactivated (5% H_2O) alumina has also been employed for the cleanup of sediment extracts (Goerlitz and Law, 1974; Wegman and Hofstee, 1982; Ernst et al., 1976). Hexane elution can be used to remove all the chlorinated hydrocarbons from the column but a further fractionation (using a Florisil or a silica column) is then required to separate the chlorinated hydrocarbons into groups suitable for GC/EC analysis.

Silica gel has higher selectivity than alumina and with proper choice of solvents, it will fractionate the chlorinated hydrocarbons. Silica fractionation can be employed in the analysis of samples containing both PCBs and a range of chlorinated hydrocarbon pesticides. Using hexane and benzene as eluents (Goerlitz and Law, 1974) or petroleum ether and 50:50 petroleum ether:methylene chloride as eluents (Wegmen and Hofstee, 1982), PCBs can be separated from most chlorinated hydrocarbon pesticides, facilitating the GC/EC quantification step.

Osadchuk and Bruns (1971) evaluated the cleanup properties of Florisil, alumina and silica gel and concluded that although they all exhibited similarities in elution patterns partially deactivated Florisil gave superior cleanup of fats and oils as compared to activated Florisil, alumina or silica. The advantage of using an alumina column for extract cleanup is that it is an excellent adsorbent for lipid material and is most easily prepared in a reproducible state of activation. However, it exhibits poor selectivity and must usually be followed by a further fractionation on silica or Florisil. Although silica exhibits good selectivity and is used to separate PCBs from chlorinated hydrocarbon pesticides, it is not very effective in removing lipids and its performance is very dependent on the degree of activation of the column (which varies from batch to batch and with the quantity of water adsorbed).

Fractionation of chlorinated hydrocarbons in extract solutions has also been carried out by use of a charcoal column (Teichman et al., 1978). Optimum separations and recoveries have been reported when the charcoal is adsorbed on the surface of an inert matrix such as polyurethane foam (Huckins et al., 1978; Chau and Babjak, 1979). Although use of this column has not been widely reported in the literature, it is successfully employed for the fractionation of sediment extracts in the laboratories at the Canada Centre for Inland Waters (Lee et al., 1982). A charcoal foam column has been used to separate PCBs from mirex and photomirex (Chau, 1980). These compounds all elute in the first Florisil fraction and can make quantification by gas chromatography difficult.

3.8.5.3 Gel Permeation Chromatography

Gel permeation chromatography (GPC) is a technique in which the separation mechanism is based on differences in molecular size. Bio-Beads SX-2 or SX-3 or Sephadex LH20 have been used with a variety of organic solvents. Largest molecules are eluted first, smallest molecules are eluted last. The molecular weights of most chlorinated hydrocarbons are in the range 200 to 500, while most lipid molecules are between 600 to 1500. GPC can therefore effect a separation of lipids from chlorinated hydrocarbons (Kuehl and Leonard, 1978; EPA, 1980). Stalling et al. (1972), in the analysis of fish extracts, used GPC columns of Bio-Beads SX-2 to effeciently (> 99.5%) remove lipids from extract solutions while recovering 100% of the chlorinated hydrocarbons. However, GPC is not effective in fractionating the chlorinated hydrocarbons and the extract solution must still be fractionated by the appropriate means (discussed above) before GC/EC quantification. The strength of the GPC procedure therefore lies in its ability to clean extract solutions high in lipid content, such as biota extracts. It has limited applicability to sediment extract solutions unless they also are heavily contaminated with lipids.

A second major advantage of this technique is that it can be fully automated, but a large number of samples would be required to justify the expense of an automated GPC system. GPC cleanup is economical as columns can be re-used.

3.8.5.4 Removal of Elemental Sulfur

Elemental sulfur is encountered in most sediment samples. The solubility of sulfur in various solvents is similar to the solubility of the chlorinated hydrocarbons, therefore the sulfur interference follows with the chlorinated hydrocarbons through the extraction and cleanup procedures. The sulfur will interfere in the GC/EC quantification step by masking the region of the chromatogram where many of the chlorinated hydrocarbon pesticides occur.

Sulfur must therefore be separated from the extract solutions before they are subjected to GC/EC analysis. This is accomplished by shaking the extract solution with a reagent which will cause the sulfur to precipitate. Several such reagents have been used:

(1) Elemental mercury (Goerlitz and Law, 1971).
(2) Activated copper powder (Plumb, 1981; Blumer, 1957).
(3) Tetrabutylammonium sulfite (Jensen et al., 1977; Teichman et al., 1978).
(4) Ethanolic potassium hydroxide (Young and Burke, 1972)
(5) Potassium cyanide (Mattsson and Nygren, 1976).
(6) Sulfuric acid (Mattsson and Nygren, 1976).

Although all of these reagents will eliminate the sulfur interference problem, they differ in their effects on the various chlorinated hydrocarbons in the extract. PCBs have been found to be stable to treatment by mercury (Chau and Lee, 1980; Addison and Nearing, 1982), activated copper powder (Chau and Babjak, 1979; Plumb, 1981), ethanolic potassium hydroxide (Addison and Nearing, 1982) and tetrabutylammonium sulfite (Bellar et al., 1980). Chlorinated hydrocarbon pesticides have also been found stable to treatment by mercury (Goerlitz and Law, 1974) and activated copper (Lee et al., 1982). However sulfuric acid, potassium cyanide, alcoholic potassium hydroxide or tetrabutyl-ammonium sulfite treatment cause degradation, and therefore low recoveries, of some of the chlorinated hydrocarbon pesticides (Mattsson and Nygren, 1976; Teichman et al., 1978; Lee et al., 1982). Therefore only the elemental mercury and activated copper powder treatments are suitable for general use. Of the two, the activated copper treatment is the method of choice since it gives better recoveries of pesticides and is less toxic than mercury (Lee et al., 1982). In addition, several repetitions of the mercury treatment may be required to completely eliminate the sulfur interference (Oliver and Bothen, 1982), while only one copper treatment is sufficient if the copper is finely powdered and activated (Lee et al., 1982).

3.8.5.5 Evaporation of Solvent

Excess solvent must be removed from the extract solution both prior to the cleanup procedure and again as the last step before GC/EC quantification. Solvent is usually removed in a Kuderna-Danish apparatus or by vacuum rotary evaporation from a low temperature bath (< 30°C) to a volume of about 2 mL. Because of the sensitivity limits of the chromatographic techniques employed for quantification and the (low) levels of chlorinated hydrocarbon usually encountered in environmental samples, extract solutions must often be evaporated further to volumes as small as 0.1 mL by evaporation in a stream of nitrogen. On this scale substantial evaporative losses of chlorinated hydrocarbons can be experienced if solvent removal is not performed with care (Lee et al., 1982). The extent of the error arising from solvent removal depends on the volatility of the solvent being removed, the particular chlorinated hydrocarbons in the extract (the more volatile pesticides such as lindane, heptachlor and aldrin are easily lost) and on the overall level of chlorinated hydrocarbons present. Significant chlorinated hydrocarbon loss has been reported for solutions evaporated to a volume of 0.5 mL or less by a stream of air or in a steam bath (Burke et al., 1966), or at room temperature on a lab bench or in a fume hood (Chiba and Morley, 1968).

This evaporative loss can be eliminated by the presence of an oil in the extract solution. If the extract solution contains oily co-extractives (such as biota extracts) or if an oily compound is added as a "keeper" before solvent removal the risk of evaporative losses can be reduced. Lee et al. (1982) studied the effect of various keepers on extracts containing fifteen different chlorinated hydrocarbons. They recommend the addition of 0.5 mL of toluene as a keeper, but caution that even then, evaporation to a final volume of less than 0.5 mL will result in significant loss of chlorinated hydrocarbons. Two methods are recommended for the evaporation of excess solvent from chlorinated hydrocarbon solutions. Evaporation on a steam bath in a Kuderna-Danish apparatus, with an attached micro Snyder column has been widely used (Burke et al., 1966; Plumb, 1981), while Chau and collaborators (Lee et al., 1982) found that simple evaporation by a gentle stream of nitrogen at 40° to 50°C gave equally satisfactory results and was more conveniently carried out.

3.8.6 Identification and Quantification of Chlorinated Hydrocarbons

Gas chromatography with either electron capture (GC/EC) or mass spectral detection with selected ion monitoring (GC/MS/SIM) is used for quantification of chlorinated hydrocarbons (Ernst et al., 1976; Castelli et al., 1983; Plumb, 1981; EPS, 1979). GC/EC is the method of choice for chlorinated hydrocarbons (Lee et al., 1982). as the electron capture detector is extremely sensitive (it responds to picogram quantities of chlorinated compounds). Although more expensive and less commonly used, GC/MS is more selective and can, at lower sensitivity, provide full mass spectral confirmation.

Gas chromatographic separation of the chlorinated hydrocarbons is achieved by use of a column containing a non-polar stationary phase. A wide range of columns has been employed including SE-30, OV-101 (Addison and Nearing, 1982), QF-1, SF-96, (FAO, 1983), OV-101, OV-17, (Castelli et al., 1983), and OV-210 (Plumb, 1981). Separation is easily achieved with packed columns operated under isothermal (approximately 200°C) conditions (Lee et al., 1982), however, capillary columns are also used when better resolution is required.

Other gas chromatographic detectors such as electrolytic conductivity and microcoulometric detectors can be used for determination of chlorinated hydrocarbons. However, they have been replaced by EC detectors because of their higher sensitivity and easier operation and maintenance procedures (Chau and Lee, 1982).

High performance liquid chromatography (HPLC) can be used to separate chlorinated hydrocarbon compounds but the technique is not widely used in the analysis of environmental samples due to the relative insensitivity of conventional HPLC detectors (e.g. UV detectors)(Lee et al., 1982; Chau and Lee, 1982).

Chemical tests such as dechlorination are used to confirm the identity of specific chlorinated hydrocarbons. Such tests are described in detail by Chau (1982).

3.8.6.1 Quantification of Chlorinated Hydrocarbon Pesticides

The identification of individual chlorinated hydrocarbon pesticides is carried out by comparison of the chromatogram of an extract solution with chromatograms of standard solutions of known pesticides (Plumb, 1981). The relative retention time of an unknown peak must match the relative retention time of a known chlorinated hydrocarbon on at least two different chromatography columns for the identity of the unknown to be confirmed (Eisenreich et al., 1980). For reference, Lee et al., 1982, have tabulated the relative retention times of some chlorinated hydrocarbon pesticides on five different gas chromtography columns. Quantification of the pesticides is accomplished by comparing the peak height of the component in the chromatogram of the extract solution with the peak height of the same component in the chromatogram of the standard solution (Ernst et al., 1976; Harvey et al., 1974). A response factor is required for each component of the extract solution since the electron capture detector exhibits a different response for each individual compound (Webb and McCall, 1973).

Chlorinated hydrocarbon pesticides can also be quantitated by GC/MS using the selected ion monitoring (SIM) technique (Onuska et al., 1980).

3.8.6.2 Quantification of PCBs

The identification and quantification of PCBs is complicated because PCBs are manufactured and used only as technical formulations containing a large number of individual compounds each with its own response factor (Hutzinger et al., 1974). Packed column gas chromatograms of PCB solutions contain approximately twenty individual peaks (Webb and McCall, 1973) and capillary column chromatograms may contain many more. Three approaches to the quantification of extract solutions containing PCBs have been reported: (1) quantification in terms of technical formulation equivalents, (2) individual component determination and (3) quantification after perchlorination. These methods have been compared critically in a review by Reynolds (1983).

a) Technical Formulation Equivalent

The technical formulation equivalent method is based on a comparison of peaks with corresponding retention times in chromatograms of the sample and of a particular technical formulation (Ernst et al., 1976; Marchand et al., 1976; Harvey et al., 1974, Chau and Babjak, 1979; Bellar et al., 1980; Galloway et al., 1983). The choice of technical formulation is based on the closeness of fit between its chromatogram and the chromatogram of the sample being analyzed. The sum of the heights of several (3 to 7) peaks in the sample chromatogram is compared to the sum of the heights of the same peaks in standard solutions of the chosen technical formulation to determine the overall level of PCBs in the unknown. This result is reported as a weight percent of the particular technical formulation in the sample or as PCBs.

Factors contributing to uncertainty and bias in the technical formulation equivalent quantification method have been discussed in detail by Duinker et al., (1980). The main problem arises from trying to compare PCBs from an environmental sample with those in one particular technical formulation. Transport (physical, chemical and biological), transformation and degradation processes in the environment have different effects on the behavior of each individual component, and the composition of PCBs in an environmental sample may be unlike that in any technical formulation. It may also originate from several sources and be a mixture of more than one technical formulation. In addition, particularly when using packed chromatography columns which provide little resolution of the components in a PCB mixture, each individual peak in the chromatogram may be due to more than one PCB component. In such a case accurate quantification of that peak in the unknown sample is impossible because the electron capture detector responds differently to each individual compound.

Only the most intense chromatographic peaks are chosen for use in the quantification. As the response of the electron capture detector is strongly dependent on the number of chlorine atoms in the compound, the less intense (and ignored) peaks may be due to components with small numbers of chlorine atoms but may be major contributors to the PCB level in the sample. The choice of technical formulation for use as the standard requires a subjective decision as standard and sample chromatograms seldom match exactly.

The main advantage in using this method of quantification is that it can be carried out on chromatographs using only packed columns (older instruments unable to accept capillary GC columns can be used). The calibration and calculation steps are easily accomplished and therefore the method is most suitable for a semi-quantitative screening of a series of samples. This method of quantification can be used with either GC/EC or GC/MS/SIM data.

b) Webb and McCall Method

This method of quantification (individual component determination) was developed by Webb and McCall (1973) and has been applied to extracts of sediment (Chau and Lee, 1980) and biota (Eder,1976; Calambokidis et al., 1979). Individual PCB components in a sample are quantified by comparison of their GC peak heights to the peak heights of the same component in a standard solution. The PCB content of the sample is determined by summing the concentration of all the individual PCB components. Although this method is often called quantification by homolog series analysis, this is a misnomer as the individual chlorobiphenyls do not constitute a homologous series. This quantification technique is useful for PCBs in environmental samples where some degradation or metabolism of PCBs is evident or where the PCBs may be a mixture of more than one technical formulation (Reynolds, 1983).

The required standard solution is a mixture of three technical formulations (usually Aroclor 1242, Aroclor 1254 and Aroclor 1260). The weight of PCB responsible for each GC peak of this standard must be known. This is calculated from knowledge of the empirical formula of the compound giving rise to each peak and the absolute amount of chlorine represented by the peak. The empirical formula can be determined from a gas chromatography/mass spectral (GC/MS) analysis and the chlorine content from the response of an electrolytic conductivity detector (response linearly proportional to the number of chlorine atoms). Preparation of a standard solution for homolog quantification is, therefore, time consuming and requires specialized equipment. However once prepared, the standard can be used indefinitely if stored properly. Some analysts use the data of Webb and McCall (1973) to calculate the concentration of their standards (Calambokidis et al., 1979) but this assumes that any batch of a particular Aroclor solution is identical to that used by Webb and McCall in 1973.

Individual component quantification of PCBs can be carried out using standards of individual polychlorobiphenyls by either GC/EC (Kerkhoff et al., 1982) or by GC/MS with selected ion monitoring (Castelli et al., 1983).

The individual component method produces the most accurate results when carefully executed (Duinker et al., 1980). However it requires well resolved chromatograms and is therefore best carried out on data obtained from capillary GC column analyses. Calibration can be time consuming and requires sophisticated instrumentation but no subjective matching of standards and samples is required and no peaks in the chromatogram are ignored.

c) Perchlorination

Quantification after perchlorination is the least used of the three methods. The PCBs in the sample and in standard Aroclor 1254 solutions are all converted to decachlorobiphenyl by perchlorination (DeKok et al., 1982; Kerkhoff et al., 1982; Duinker et al., 1980). The resultant solution gives only one GC peak which is easily detected by the electron capture detector. Comparison of the height of the decachlorobiphenyl peak in the perchlorinated sample and standard solutions is used to determine the level of total PCBs in the sample which are reported as % Aroclor 1254.

The perchlorination method appears to produce higher results than the other two methods of quantification (Duinker et al., 1980; Kerkhoff et al., 1982; DeKok et al., 1982). Possible reasons for this have been discussed in detail by Duinker et al., 1980, but the main cause seems to be poor matching of the environmental sample to the standard sample. They report that individual PCB components are converted to decachlorobiphenyl with different efficiencies (ranging from 60 to 100%) depending on the initial degree of chlorination of the molecule. Therefore PCBs in poorly matched sample and standard solutions may be perchlorinated to different extents causing errors in the final results. The advantage of this method is the simplification of the chromatographic quantification of the PCBs, but this is likely more than offset by the extra sample handling required to perchlorinate the PCBs to decachlorobiphenyl. This method would only be suitable for a semi-quantitative screening of samples for overall PCB level.

The advantages and disadvantages of the three quantification methods are outlined in Table III-3.31. The Webb and McCall method produces the most information about the PCBs in the sample (level of each component as well as overall level is obtained), but it requires sophisticated instrumentation, elaborate calibration and is the most time consuming.

3.8.7 Summary

Interlaboratory comparisons of the analysis of chlorinated hydrocarbons in sediment (Pavlou and Hom, 1976; Guevremont et al., 1982), and biota (Harvey et al., 1974; Martin et al., 1982; Galloway et al., 1983), are summarized in Table III-3.32. These comparisons indicate a large variability in the results reported by various laboratories. The many steps required to analyze chlorinated hydrocarbons are each a potential source of variation. Each laboratory employs its own combination of extraction, cleanup and quantification procedures to the extent that no two laboratories perform exactly the same analysis procedures from start to finish. This makes detection of the exact source of variability in the interlaboratory studies impossible.

Time per analysis of sediment or biota samples for chlorinated hydrocarbons is in the range of eight hours to four days, dependent mainly on the

TABLE III-3.31

COMPARISON OF THE METHODS USED TO QUANTIFY PCBs IN EXTRACT SOLUTIONS

METHOD	ADVANTAGES	DISADVANTAGES
Technical Formulation Equivalents	- requires only basic GC/EC instrumentation with packed column capability - easiest and fastest of the three methods	- requires matching of chromatograms of sample and commercial technical formulations (never exact) - results can be biased in favour of the level of the more chlorinated components, as many peaks in the chromatogram are ignored - no information obtained on individual PCBs present or their concentration - separate standard choice may be required for each sample analyzed
Webb and McCall Method	- each PCB component is identified and quantitated individually - no choice of standard technical formulation is required - most accurate results obtained	- calibration is time consuming and requires sophisticated instrumentation (GC/MS)
Perchlorination	- chromatographic interpretation is simplified - calibration is simplified	- perchlorination requires extra sample handling providing extra source of error - efficiency of perchlorination of individual PCB components is variable - produces higher results than other methods - no information obtained on individual PCBs present or their concentration

TABLE III-3.32

DATA VARIABILITY IN INTERLABORATORY CALIBRATION STUDIES

REFERENCE	NO. OF PARTICIPANTS	SAMPLE	MEAN	% RELATIVE STANDARD DEVIATION
Harvey et al., 1974	6	PCBs in biota	12.5 $\mu g \cdot g^{-1}$	21
	5	ΣDDT in biota	3.4 $\mu g \cdot g^{-1}$	27
Pavlou and Hom, 1976	10	PCBs in sediments	612 $ng \cdot g^{-1}$	22
	6	ΣDDT in sediments	31 $ng \cdot g^{-1}$	90
Guevremont et al., 1982	7	PCBs in sediments	38 $ng \cdot g^{-1}$	140
	7	PCBs in sediments	4.0 $ng \cdot g^{-1}$	49
Galloway et al., 1983	5	PCBs in biota	510 $ng \cdot g^{-1}$	38

extraction technique chosen (Soxhlet extractions may require seventy-two hours, other extractions can be carried out within an hour). However, a turnaround of about two samples per analyst per day can be expected. The methods usually used require commonly available glassware and reagents and the use of a gas chromatograph with either an electron capture or mass spectrometer detector.

Reference and intercalibration materials for chlorinated hydrocarbon pesticides and PCB analyses are listed in Table III-3.33.

TABLE III-3.33

CHLORINATED HYDROCARBON REFERENCE AND INTERCALIBRATION MATERIALS

I.D.	SOURCE	CERTIFIED COMPONENTS	COMMENTS
CS-1	NRC Canada	PCBs (Aroclor 1254)	Coastal marine sediment dried, homogenized
HS-1	NRC Canada	PCBs (Aroclor 1254) 10 individual chlorobiphenyls	Harbour sediments
HS-2	NRC Canada	PCBs (Aroclor 1254) 10 individual chlorobiphenyls	Harbour sediment
EC-1	CCIW	Organochlorine compounds PCBs chlorobenzenes	In preparation Lake sediment
EC-2	CCIW	Organochlorine compounds PCBs chlorobenzenes	In preparation Lake sediment
MA-A-1	IAEA	5 chlorinated pesticides 2 PCB (Aroclor 1254)	Copepod Homogenate 15 lab intercalibration
MA-M-2	IAEA	7 chlorinated pesticides 2 PCBs (Aroclor 1254,1260)	44 lab intercalibration
Capelin Oil	ICES	7 chlorinated pesticides 1 PCB (Aroclor 1254) (21 compounds identified)	44 lab intercalibration 3rd ICES Intercalibration sample
Mussel	ERNL/USEPA	PCB (Aroclor 1254) DDE	Intercalibration sample

3.8.8 Literature Cited

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4. MEASUREMENT OF SEDIMENT GRAIN SIZE

4.1 Introduction

Grain size is an important and fundamental sediment property that has application in a number of areas. Grain size distributions offer information on despositional environments, sedimentation processes and on the geotechnical properties of the bulk sediment. In environmental studies, sediment grain size is important in comparing and evaluating chemical composition and benthic biota. Many environmental pollutants are scavanged from the water column through sorption processes with settling particulates. The effective surface area of particulates increases in inverse proportion to particle size so that the finer particulates have a much larger effective surface area for a given weight of material. The finer size particulates also include mineral phases with a higher trace metal content than those of the coarser material (Calvert, 1976). As a result almost all trace metals and organics are partitioned into the fine size range (< 63μm) and are not evenly distributed within the sediment matrix. A comparison of the chemical composition of sediments and associated benthic communities therefore must be made on the basis of grain size characteristics.

It is usual to describe sediments on the basis of their gross grain size characteristics as gravel, sand, silt, clay or combinations thereof. These terms correspond to a broad range of particle sizes (generally taken as mean particle diameter) ranging from less than a micron to several centimetres. In North America, the Wentworth classification system is widely used to describe grain size characteristics of aquatic sediments. Other systems are used in soil science and in Europe (Head, 1981). The definition of sediment types in terms of particle diameter using the Wentworth classification is shown in Table III-4.1.

Actual methods for grain size analysis measure quite different properties of individual particles which relate in various ways to actual size. Sieving techniques are the most popular method and discriminate sediment particles on the basis of their smallest cross-sectional diameter. Settling tube or sedimentation methods measure the settling velocity of particles in water. Electronic particle counting methods determine particle size on a volume basis. Microscopic analysis of fines gives measurements which are most directly related to actual grain size as well as information on grain shape.

TABLE III-4.1

RELATIONSHIP BETWEEN VARIOUS SIZE UNITS, U.S. STANDARD SIEVE SIZES AND WENTWORTH CLASSIFICATION

	U.S. STANDARD SIEVE MESH	MILLI-METERS	PHI (ϕ) UNITS	WENTWORTH SIZE CLASS	COMMON TEST PROCEDURES
GRAVEL	5	16	-4	Pebble	Sieve analysis
	-	4	-2		
	6	3.36	-1.75		
	7	2.83	-1.50		
	8	2.38	-1.25	Granule	
	10	2.00	-1.0		
SAND	12	1.68	-0.75		
	14	1.41	-0.5	Very Coarse Sand	
	16	1.19	-0.25		
	18	1.00	0.0		
	20	0.84	0.25		
	25	0.71	0.5	Coarse Sand	Settling Tube or Sieve
	30	0.59	0.75		
	35	0.50	1.0		
	40	0.42	1.25		
	45	0.35	1.5	Medium Sand	
	50	0.30	1.75		
	60	0.25	2.0		
	70	0.210	2.25		
	80	0.177	2.5	Fine Sand	
	100	0.149	2.75		
	120	0.125	3.0		
	140	0.105	3.25		
	170	0.088	3.5	Very Fine Sand	
	200	0.074	3.75		
	230	0.0625	4.0		
SILT	270	0.053	4.25	Very Coarse Silt	Sedimentation techniques: Pipette, Hydrometer; Optical Sensors X-ray
	325	0.044	4.5		
	-	0.037	4.75	Coarse Silt	
	-	0.031	5.0		
	-	0.0156	6.0	Medium Silt	
	-	0.0078	7.0	Fine Silt	
	-	0.0039	8.0	Very Fine Silt	
CLAY	-	0.0020	9.0		
	-	0.00098	10.0	Clay	
	-	0.00049	11.0		Practical Limit of Sedimentation Analysis
	-	0.00024	12.0		
	-	0.00012	13.0		
	-	0.00006	14.0		

$\phi = -\log_2$ diameter (millimeters)

No one method is capable of measuring grain size over the full range of gravel to clay size particles. The methods of choice will therefore depend on:

1. requirements of the data and the detail desired;
2. the size of the largest particle present;
3. the range of particle sizes present; and
4. facilities and funds available.

For a detailed analysis of sediments with a broad range of particle size, at least two methods will be required. Traditionally, sieving methods have been used to separate particles in the sand and gravel size categories (down to 63 μm) while pipet and hydrometer sedimentation techniques have been used for size measurements of the clay and silt fractions. Sedimentation techniques can also be used for sand analysis (particles to 2 mm) and numerous automated methods have been developed using this principle.

Electronic particle counting techniques have a wide theoretical operating range. These methods have become fairly standardized because of the available instrumentation and are a recommended method of the EPA (Plumb, 1981). Sieve, sedimentation and electronic counting techniques are reviewed in the following sections. Microscopic procedures have not been included because of their limited use and expensive instrumentation requirements. Further information on these techniques can be found in Swift _et_ _al_., (1972).

4.2 Sieving Techniques

Sieve analysis of sediment size has a long history and is by far the most frequently used technique for size analysis. Its popularity is due to the relatively low cost and simplicity. Although sieves down to 5 μm are available, a practical limit to sieve analysis is about 50 μm. Sieve techniques have been standardized and very detailed protocols developed for sample preparation, sieving technique and data handling (Walton, 1978; Krumbein and Pettijohn, 1938; Carver, 1971; Head, 1981). Sengupta and Vienstra (1968) found the error in the sieve analysis of fine sand sized particulates (based on spherical glass beads) to be about 18% while the reproducibility was between 0.4 and 1.3% depending on size. The main sources of variability in the results are:

1. shaking time
2. sample size
3. sample preparation
4. irregularities in grain shape
5. deformation in the sieve aperatures

Shaking times are a compromise between wear on the sieve and efficiency both of which increase with time. Shaking times of 15-25 minutes appear to be optimal (Griffiths, 1967). Too much sample will mean some particles will fail to contact the sieve mesh while too little will give an insufficient amount for all sieve fractions. Sample preparation is important as the presence of organic material and large quantities of fines will make the sediment more cohesive. As a result, fines are usually removed by wet sieving and organics destroyed by oxidation with H_2O_2 (see section 4.5). After removal of fines and organics, the sediment is dried before sieving. Disaggregation procedures will obviously affect the result (Nelsen, 1983). These potential problems do not however, appear to contribute significantly to the total error in sieving carried out in a standardized manner. Manufacturing defects in sieve aperture size in the study by Sengupta and Vienstra (1968) accounted for 95% of the total error.

The chief drawback in sieving techniques is the time required for analyses. Detailed sieve analysis of sand and gravel may require up to four hours per sample.

4.3 Sedimentation Techniques

Sedimentation techniques for estimating grain size are based on the assumption that the settling velocity of particles in a liquid is a function of size, large particles settling faster than smaller ones of similar density and shape. The maximum or terminal velocity of a given particle can be related to particle diameter by Stokes' Law (terminal velocity is proportional to the square of particle diameter for spherical particles).

In practice, a suspension of a known mass of fine sediment is made up in a known volume of water. The particles are allowed to settle under the force of gravity in a column and by applying Stokes' Law, the maximum diameter of particles remaining at a particular depth after a certain length of time can be calculated.

There are many complicating factors in all sedimentation methods and a far greater degree of control is required compared to sieve methods. Settling velocity is a function of the interaction of three interdependent particle properties (mass, shape and size). To derive particle size from settling velocity using Stoke's Law, the following assumptions are made:

1. there is no turbulence within the water column
2. particles are spherical
3. terminal velocities are small
4. particles have the same density

As particles settle through the column they create turbulence and any temperature fluctuations will cause convective currents and changes in fluid viscosity. Sedimentation methods, must therefore keep the temperature fairly constant. The assumption of similar density and shape will obviously not be valid for many sediment particles. Sedimentation methods are generally calibrated with spherical glass beads with a density of 2.65 $g \cdot cm^{-3}$. Grain size determinations made via sedimentation techniques do not therefore give true grain size but rather derive an "equivalent spherical diameter".

Besides physical properties of the particles, there are a number of design factors that modify settling velocity. These include (Amos et al., 1981; Gibbs, 1972):

1. hindered settling (particles slowed as a result of high particle concentrations with finer particles interfacing with the free fall of larger ones
2. settling induced convection resulting in increased settling velocity for fine particles
3. mass flow where grains settle faster than their normal fall velocity
4. wall induced hinderance of particles close to the settling column walls.

The first three effects arise principally from too much sample and can be mimimized by proper choice of sample size. The fourth effect can be reduced by using a tube of sufficiently large diameter (Gibbs, 1972). These factors are important when comparing data from two different sedimentation methods.

Settling methods have many inherent advantages (Taira and Scholle, 1977):

1. settling velocity has more environmental significance and is more closely related to the deposition environment than sieve size.
2. settling velocity techniques are capable of measuring a wide range of particle sizes (1 to 10^4 microns)
3. they are much faster compared to sieving techniques over similar size range
4. they require small sample size
5. they yield more continuous frequency curves

There are several means of estimating the settling velocity. The most common are: 1) by sampling from a specified depth and weighing the particles present (pipet test), 2) measuring the density of the suspension with an hydrometer (hydrometer test), 3) measuring sediment accumulation rates with a sensitive microbalance, or 4) using light or x-ray transmission.

Pipet analysis of sedimentation rate is the standard method and the most widely used. Hydrometer methods are also widely used though less precise. The popularity of these methods is due to their relative simplicity and low equipment costs. The two techniques are compared in Table III-4.2 in terms of sample requirements and performance characteristics. Detailed procedures can be found in the references.

Microbalance, optical and x-ray methods have advantages over the classical pipet and hydrometer sedimentation methods in that no sub-sampling is required that will disturb the column and the methods can be fully automated increasing the speed considerably. The first factor also means greater precision. Jordan et al. (1971) found that using a photo-extinction method gave four to five times better precision than could be obtained with pipet analysis in the silt and clay size range.

Automated sedimentation methods for the analysis of sand and coarse silt have been developed on the basis of photoextinction, light transmission and weight measurements (Taira and Scholle, 1977; Stockham and Fochtman, 1977). These methods are rapid and more precise than sieving and offer distinct advantages over sieving techniques when large numbers of samples are to be processed and sample size is limited. Complete analysis is typically 10 minutes while sieving methods will require 2-4 hours.

Most of these methods employ either a sensitive balance for measuring accumulated sediment or an optical sensor. Two methods using a balance and

TABLE III-4.2

PIPET AND HYDROMETER SEDIMENTATION TECHNIQUES

	PIPET	HYDROMETER
Sample Requirements	5 - 25 g	50 - 100 g
Practical Range of Application	0.5 - 63 μm	~ 1 - 63 μm
Time Required	almost 3 days required to carry out analyses to 0.5 μm; 17 hours to 1 μm.	slower than pipet to analysis for particle size analysis of equivalent (24 hours to 1.5 μm)
Comments	more precise than hydrometer because of less disturbance of water column. precision (CV) of approx. 0.5 Ø attainable	less precise but less exacting procedure; suitable for a field lab as no drying or weighing required
Cost	$60-120/sample depending on detail	$40-60/sample
References	Walton, 1978; Krumbein and Pettijohn, 1938; Carver, 1971	Head, 1981; Krumbein and Pettijohn, 1938

TABLE III-4.3

AUTOMATED SETTLING TUBE SYSTEMS FOR COARSE SILT AND SAND

MEASUREMENT	TUBE DIMENSION	SIZE RANGE	SENSI-TIVITY	TIME FOR ANALYSIS	SAMPLE SIZE	REFERENCE	COMMENTS
Sedimentation Balance	150 cm x 12/8 m	30 - 2000 µm	not stated	2-10 min	0.1 - 1 g	Gibbs, 1974	see figure III-4.1 not commercially available; principal costs are electro-balance and data handling; accuracy stated as better than 5% (based on spherical glass particles)
Sedimentation Balance	200 cm x 15.5 cm	50 - 1000 µm	~ 20 µm	10 minutes (including plotting, statistics)	5 - 15 g	Amos _et_ _al_., 1981	not commercially available; precision est. at 2.5 - 6.6%
Photo-Extinction	146 cm x 14 cm	30 - 2000 µm	1/18 0	not stated	0.1 - 1 g	Taira and Scholle, 1977	not commercially available; more sensitive than balance techniques;

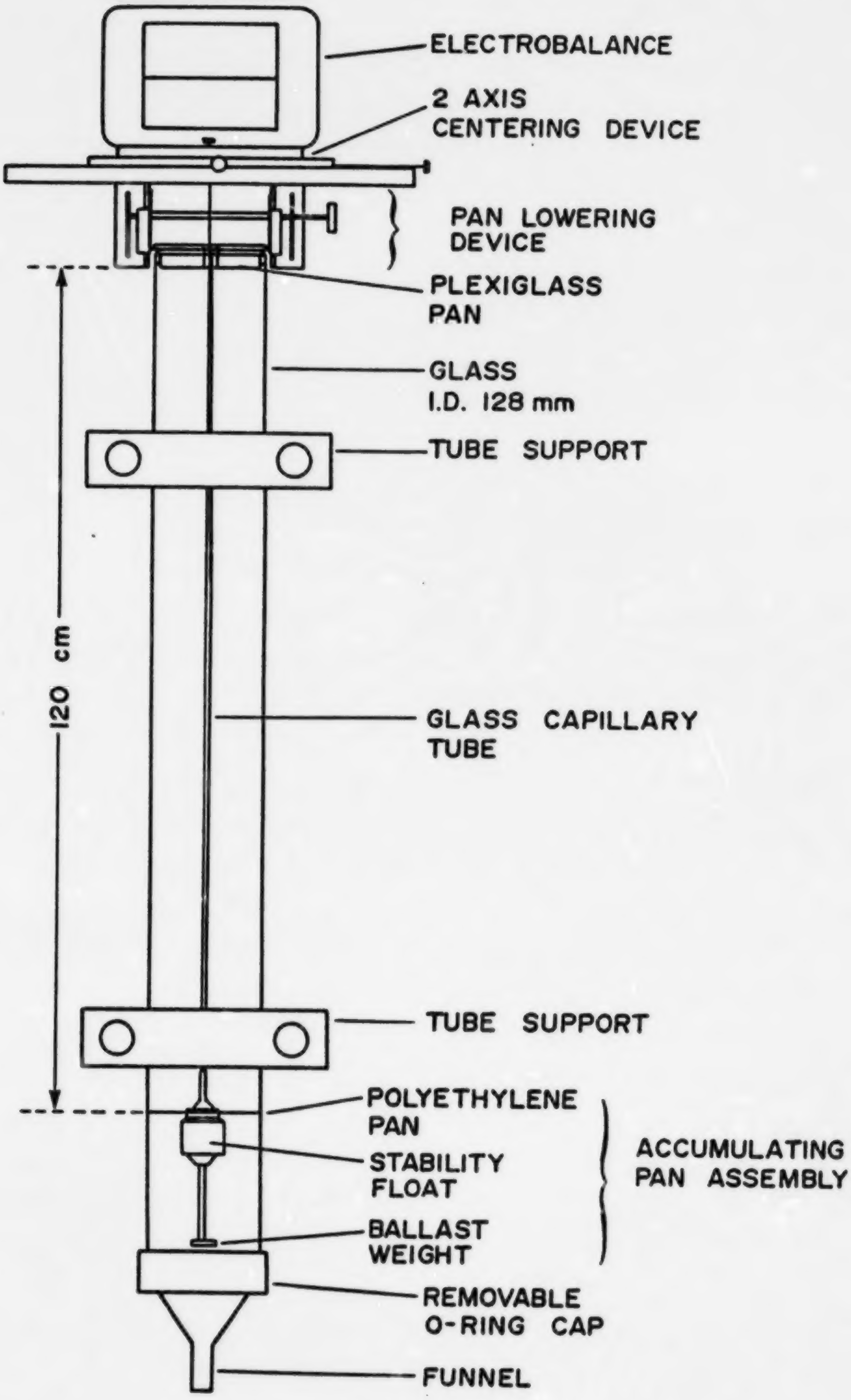
ELECTROBALANCE
2 AXIS CENTERING DEVICE
PAN LOWERING DEVICE
PLEXIGLASS PAN
GLASS I.D. 128 mm
TUBE SUPPORT
120 cm
GLASS CAPILLARY TUBE
TUBE SUPPORT
POLYETHYLENE PAN
STABILITY FLOAT
BALLAST WEIGHT
ACCUMULATING PAN ASSEMBLY
REMOVABLE O-RING CAP
FUNNEL

Figure III-4.1. An automated settling tube for sand particulate size analysis (from Gibbs, 1974).

photoextinction measurement are compared in Table III-4.3. There are a number of devices in the literature developed by various sedimentologists and research organizations. They are not usually commercially available and in terms of set-up time and materials, quite expensive. However, where large numbers of samples must be processed, the higher initial cost will be warranted.

Automated settling tubes have also been developed for the analysis of silt and clay sized particles. These employ much smaller settling chambers and usually either light or x-ray transmission for measurement. A commercially available instrument using a balance is also available. Some examples of automated methods are given in Table III-4.4. Automated methods for the analysis of clay and silt are more precise and much faster than standard pipet or hydrometer tests. However, equipment costs are high and likely prohibitive for most environmental laboratories. Sedigraphs (Table III-4.4), commercially available instruments utilizing a mini-settling tube and x-ray transmission or photo extinction for measurement of settling velocity for particles in the silt and clay size range cost between $26-40,000.

4.4 Electronic Particle Counting

This method is based on the change in electronic resistivity caused by a particle as it passes through an aperture immersed in a suitable electrolyte. The data obtained is related to particle volume rather than size as the changes induced in the elctronic field are proportional to the volume of electrolyte displaced by the particle. The method has been reviewed in detail by Sheldon and Parsons (1967). Applications to marine sediment particle counting are reviewed by Kranck and Milligan (1979). Particle diameter can be calculated assuming spherical particles. The resolution of the technique is basically 1/3 Ø. The results of 3 consecutive channels can therefore be summed to give a 1 Ø value. Good correlation has been obtained between particle sizes obtained by this method and conventional pipet analysis (Swift et al., 1972).

The advantages of this method are its speed, sensitivity, wide range (0.8 - 1200 μm), and small sample requirements. No analytical "break" results such as occurs when switching methods (sieve to pipet for example). The small sample size makes it possible to analyze very fine layers such as the surface floc or narrow layers from core samples. The instrumentation is portable, not sensitive to motion and therefore adaptable to ship board use. Another advantage of the technique is that since the data are on a volume basis, it is possible to calculate the density of the

TABLE III-4.4

AUTOMATED SEDIMENTATION METHODS FOR CLAY AND SILT-SIZE PARTICLES

MEASUREMENT	SIZE RANGE	RESOLUTION	TIME FOR ANALYSIS	SAMPLE SIZE	REFERENCE
Photo-Extinction	4 - 8 ϕ	0.2ϕ	10 min	5 - 10 g	Jordan et al., 1971
Photo-Extinction and X-Ray	0.2 - 50 μm	0.05 μm (theoretical)	10 min to 2 μm 20 min to 1 μm 100 min to 0.2 μm	2 g	Micrometritics Instrument Corporation (Sedigraph series)
Micro-Balance (semi-automated)	1 - 50 μm	< 1 μm	8 hours to 1 μm	0.1 - 2 g	Cahn sedimentation balance

particulates which can be useful in distinguishing mineral types and identifying the origins of the particulate material (organic aggregates, flocs, clays and other minerals will have quite different densities, for instance).

The sensitivity of the technique makes it prone to counting errors as gas bubbles or density changes can be mistaken for particles. These are operational problems however, that can be minimized or eliminated with proper technique. The small size also makes sample preparation more difficult as considerable dilution is required for bulk samples.

The method has been recommended by EPA as a standard method for particle size analysis (Plumb, 1981) and the widespread use and availability of counting equipment make this an attractive option. The equipment is expensive, however ($20-30,000), especially compared to pipet or hydrometer analysis, and this is a major drawback to its widespread application.

4.5 Sample Preparation for Grain Size Analysis

Samples for grain size analysis are generally stored cool in a sealed container to prevent moisture loss. Sample size will vary with the type of sediment and analysis technique. Coarse sediments will require from 50 - 100 g or more for sieve analysis, while 20 g or less will suffice for fines by pipet analysis.

Sample preparation methods are somewhat arbitrary and depend on the final use of the data, properties of the sediment and the precision required. A sedimentologist, for instance, would wish to break-up any particulate aggregates while the environmentalist may wish to know environmental particle size and therefore aggregate size. In all cases however, the goal of sample preparation is to ensure that the measurements made of size distribution reflect natural variations and are not artifacts of sample preparation (Nelsen, 1983). Nelsen emphasized that a consistent manner of sample preparation is crucial to the final interpretation of data. Depending on the sediment type and the degree of splitting or dilution required, sample preparation times may equal or greatly exceed analysis time. This is especially true in automated methods.

Walton (1978) outlined a generalized scheme that is widely used for the preparation of samples for analysis by sieve and pipette techniques (Figure III-4.2). Samples are usually wet-sieved initially through a coarse 16 mm mesh sieve to

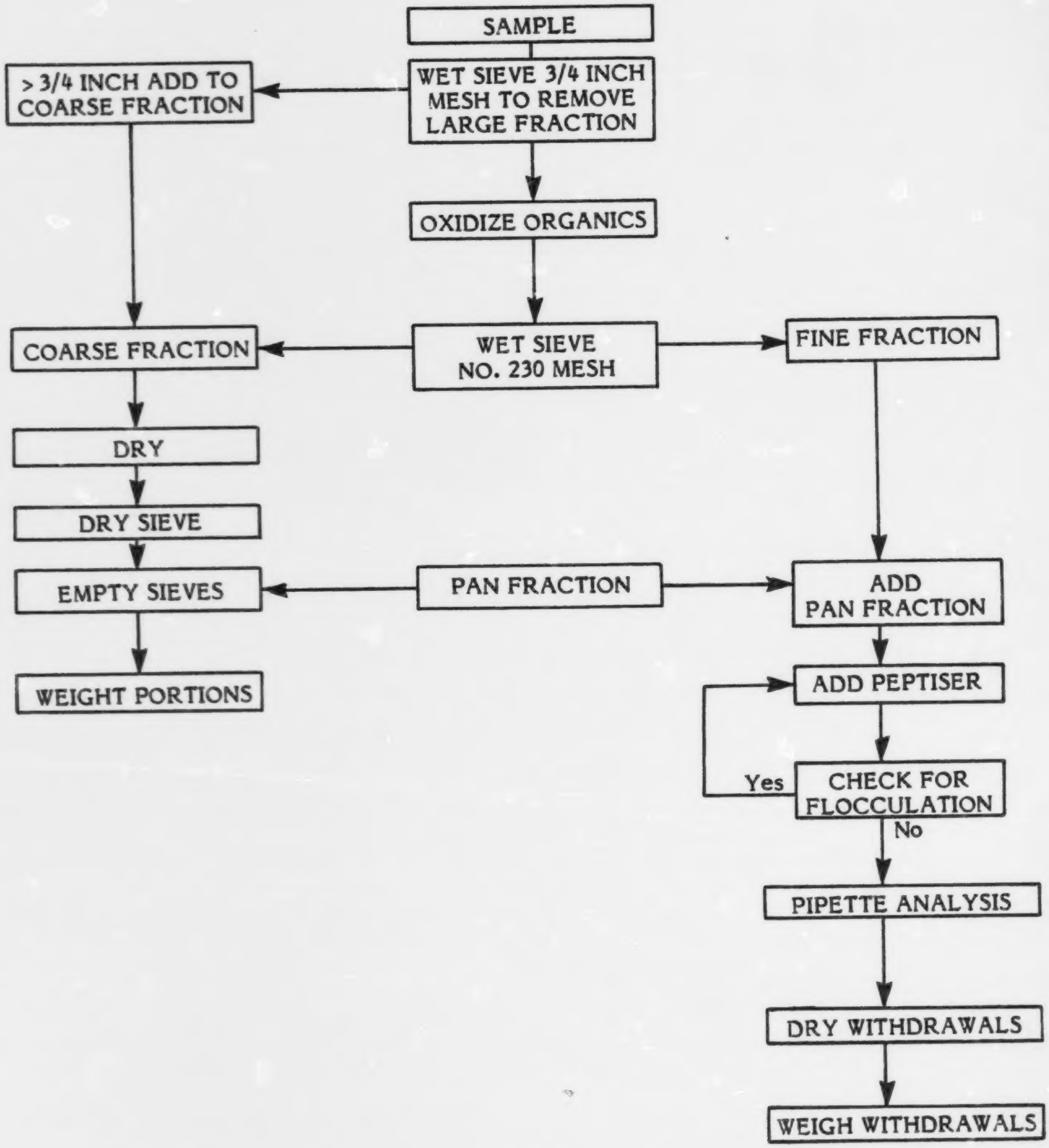
SAMPLE
> 3/4 INCH ADD TO COARSE FRACTION
WET SIEVE 3/4 INCH MESH TO REMOVE LARGE FRACTION
OXIDIZE ORGANICS
COARSE FRACTION
WET SIEVE NO. 230 MESH
FINE FRACTION
DRY
DRY SIEVE
EMPTY SIEVES
PAN FRACTION
ADD PAN FRACTION
WEIGHT PORTIONS
ADD PEPTISER
Yes
CHECK FOR FLOCCULATION
No
PIPETTE ANALYSIS
DRY WITHDRAWALS
WEIGH WITHDRAWALS

Figure III-4.2 General laboratory procedure for sediment, grain size distribution determination by sieve and pipette analysis (from Walton, 1978).

remove cobbles. The sediment passing the sieve can be treated in a number of ways before further analysis and depending on the techniques to be used. The goal of these procedures is to prevent particles from aggregating or sticking to equipment. Organic material if present in large quantities (1-2% or more) is usually removed, generally by boiling with H_2O_2 (exact procedures can be found in Walton, 1978 or Carver, 1971). Depending on the method to be used, the remaining sediment is then wet sieved to separate the "fines" from gravel and sand for sieve and pipet analysis or just to separate gravel for settling tube or electronic particle counting methods. If analysis of silt and clay size particles is required, the fines passing a 230 mesh sieve must be concentrated, either by centrifuging or allowing time for settling and then decanting excess water. Either method is time consuming especially where a large volume of water has been used in wet sieving.

For sieving, or settling tube methods the sand and gravel fraction is usually dried, weighed then disaggregated before analysis. Disaggregation is usually not a problem with clean sand and gravel.

The fine fraction if dried in an oven tends to be cohesive especially if there is a lot of clay present and the method of disaggregation critical to the final result. Freeze drying is preferable to oven drying for samples with a high clay content. Low temperature ashing is also an effective means of sample preparation (Kranck and Milligan, 1979). Nelsen (1983) compared four methods for disaggregation namely 1) soaking with Calgon, 2) shaking in water with a small amount of Calgon 3) ultrasonification and 4) stirring with a malt-mixer type stirring device. On the basis of the tests, Nelson reached the following conclusions:

1. Under similar conditions of disaggregation and analysis there is no statistically significant difference between the size distributions of freeze or air-dried portions of a given sediment.

2. The disaggregation time needed to arrive at the terminal distributions seem to partition the methods into two groups, the very rapid (stir and ultrasonify) and the variable (shake and soak) disaggregation times.

3. From the data available the methods with variable disaggregation times seem to be sample dependent.

4. For a given sample the terminal distributions produced by all the disaggregation methods were statistically indistinguishable at the 95% confidence level.

The addition of a dispersant is usually required. The most commonly used dispersant is sodium hexametaphospate (available commercially as "Instant Calgon") which is added to a suspension of the fines in sufficient quantity to inhibit aggregation and accelerated settling velocities.

Techniques using very small samples such as electronic counting and automated settling tube methods require more care in sample preparation as the population size is much smaller. This will involve microsplitting of samples to obtain representative subsamples which is more time consuming than subsampling for much larger weights.

4.6 Data Presentation

The presentation of grain size can be accomplished in a number of ways depending on the use and detail required. As a minimum, data should be tabulated so that actual weights used and weights in various size fractions are given. In the case of settling tube methods, raw data on timing and method of calculation should be presented as this information along with a detailed description of methods is important in evaluating data for comparative purposes. There are numerous methods of presenting data in support of sedimentological studies (Till 1974, Carver 1971, Doeglas 1968). However, grain size data in support of environmental studies does not usually require the same detail. As the primary purpose of grain size distribution will be to define the primary sediment characteristic and for comparisons of sediment types, grain size data is usually presented in some sort of graphical form. Three ways of doing this are presented here.

When a series of measurements have been made, the most common approach is to plot grain size (on a log scale) vs the % of material finer or coarser than the total and by assuming a continuous distribution, create a cummulative frequency distribution curve (see Walton, 1978). An example is shown in Figure III-4.3 for some hypothetical results given in Table III-4.5. This method has the advantages of wide use (standardized engineering format) and ease of interpretation. At the same time, it is possible to compare several different sediments on one graph.

Alternately, data may be plotted as a histogram relating size range vs amount of material. This form of presentation has the advantage that it corresponds more directly to the actual measurements which are made at discrete intervals and are not continuous. Comparing different sediments on one plot, however, is more difficult (Till, 1974; Stockham and Fochtman, 1974).

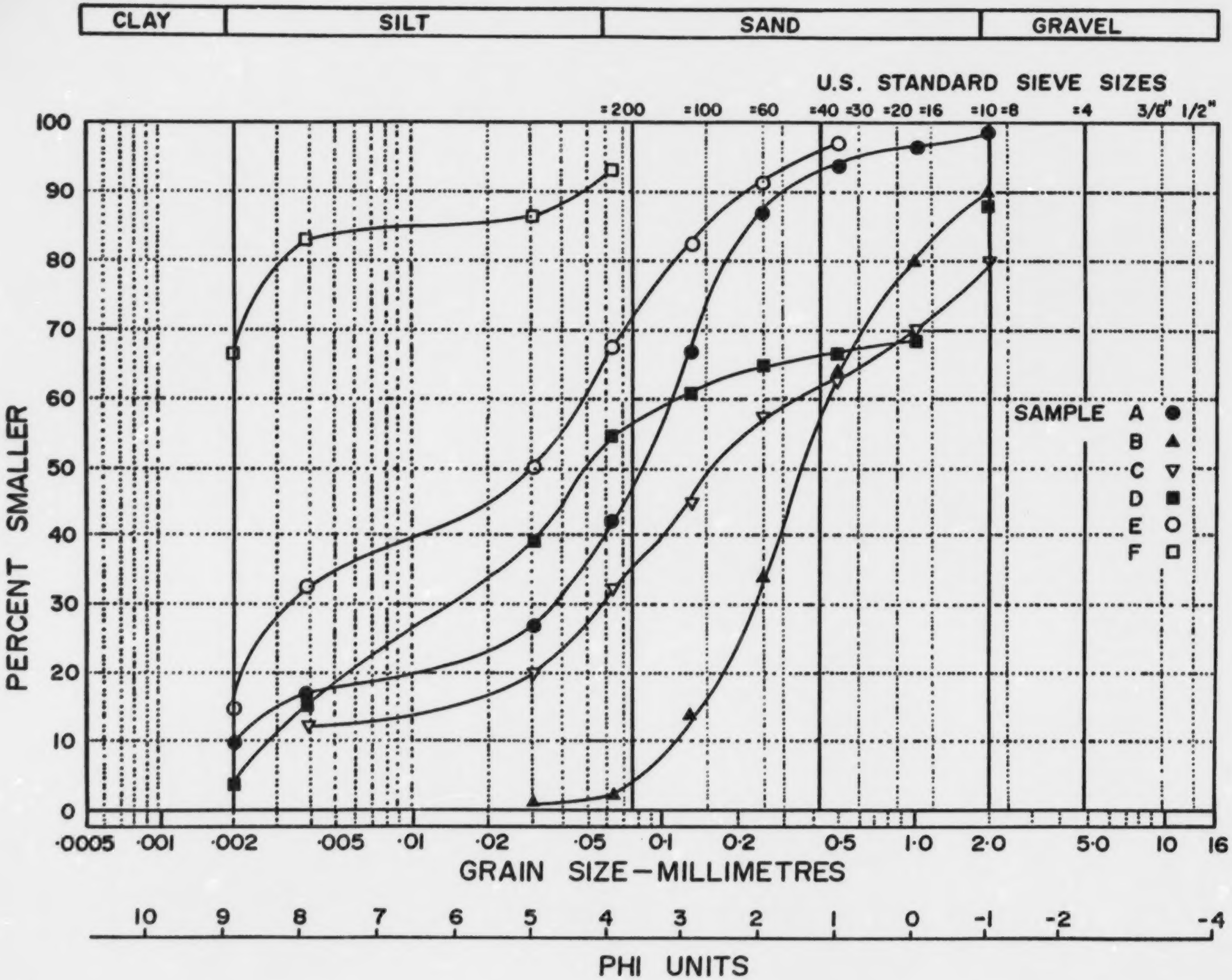

Figure III-4.3. Cumulative frequency distribution plot of grain size. (see Table III-4.5 for sample grain-size data).

TABLE III-4.5

EXAMPLE OF GRAIN SIZE DISTRIBUTION DATA

Sieve	Diameter	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F	
		Weight (g)	% Finer Than	Weight (g)	% Finer Than	Weight (g)	% Finer Than	Weight (g)	% Finer Than	Weight (g)	% Finer Than	Weight (g)	% Finer Than
10	- 1.0	0.5	98.25	5.0	90	8	80	3	88.2	-	100	-	100
18	0.0	1.0	96.25	5.0	80	4	70	5	68.6	-	100	-	100
35	1.0	1.2	93.25	8.0	64	3	62.5	0.5	66.6	1	97.1	-	100
60	2.0	2.5	87	15.0	34	2	57.5	0.5	64.6	2	91.2	-	100
120	3.0	8	67	10.0	14	5	45	1	60.7	3	82.4	-	100
230	4.0	10	42	6	2	5	32.5	1.5	54.8	5	67.7	1.0	93.3
-	5.0	6	27	0.5	1	5	20	4	39.1	6	50.1	1.0	86.6
-	8.0	4	17	0.5	0	3	12.5	6	15.6	6	32.5	0.5	83.3
-	9.0	3	9.5	-	-	1	10.	3	3.8	6	14.9	2.5	66.6
-	> 9.0	3.8	-	-	-	4	0	1	0.	5	0	10.0	0
Total Weight (g)		40.0		50.0		40.0		25.5		34.0		15.0	

Note: Above data is presented in Figures III-4.1 and III-4.2.

Another common means of presenting grain size data is via a triangular classification (Shepard, 1954; Folk, 1954). The percent composition expressed in terms of any 3 characteristics can be plotted. Usually the sand, clay, silt or sand plus gravel, clay, silt ratios are used. Any three end members could be chosen, however, depending on the sediment characteristics. This also provides a definition for a description of the sediment (ie. clay, clayey sand etc). This is the best method for comparing a large number of sediment samples at one time (Figure III-4.4). These are by no means the only ways of presenting data, but because of their widespread use, are recommended for ease of comparison with historical data.

4.7 Summary

The methods reviewed here are compared below in terms of initial costs, applicable size range, sample requirements and approximate time for analysis. (Note that costs are estimates only).

METHOD	INITIAL EQUIPMENT COST	SIZE RANGE µm	SAMPLE REQUIRE-MENTS (dry weight)	TIME (per sample)*
Sieve	$ 500 +	30 - 16000	40-150 g	2-4 hrs
Pipet (+ hydrometer sedimentation)	$ 500 - 1000	0.5 - 62	1-5 g	up to 60 hrs
Settling Tube (automated)	$5,000 +	30 - 2000	0.1 - 15 g	2-10 minutes
Coulter Counter	$20,000 - 30,000	0.3 - 800	< 1 g	10 minutes
(Sedigraph)	$35,000 (x-ray)	0.2 - 50	2 g	10-100 minutes

* does not include sample preparation time. This increases with decreasing sample size and with the heterogeneity of the sediment. Sample preparation time is generally the most time consuming step in Coulter Counter or automated settling tube methods. Preparation time may be several hours per sample.

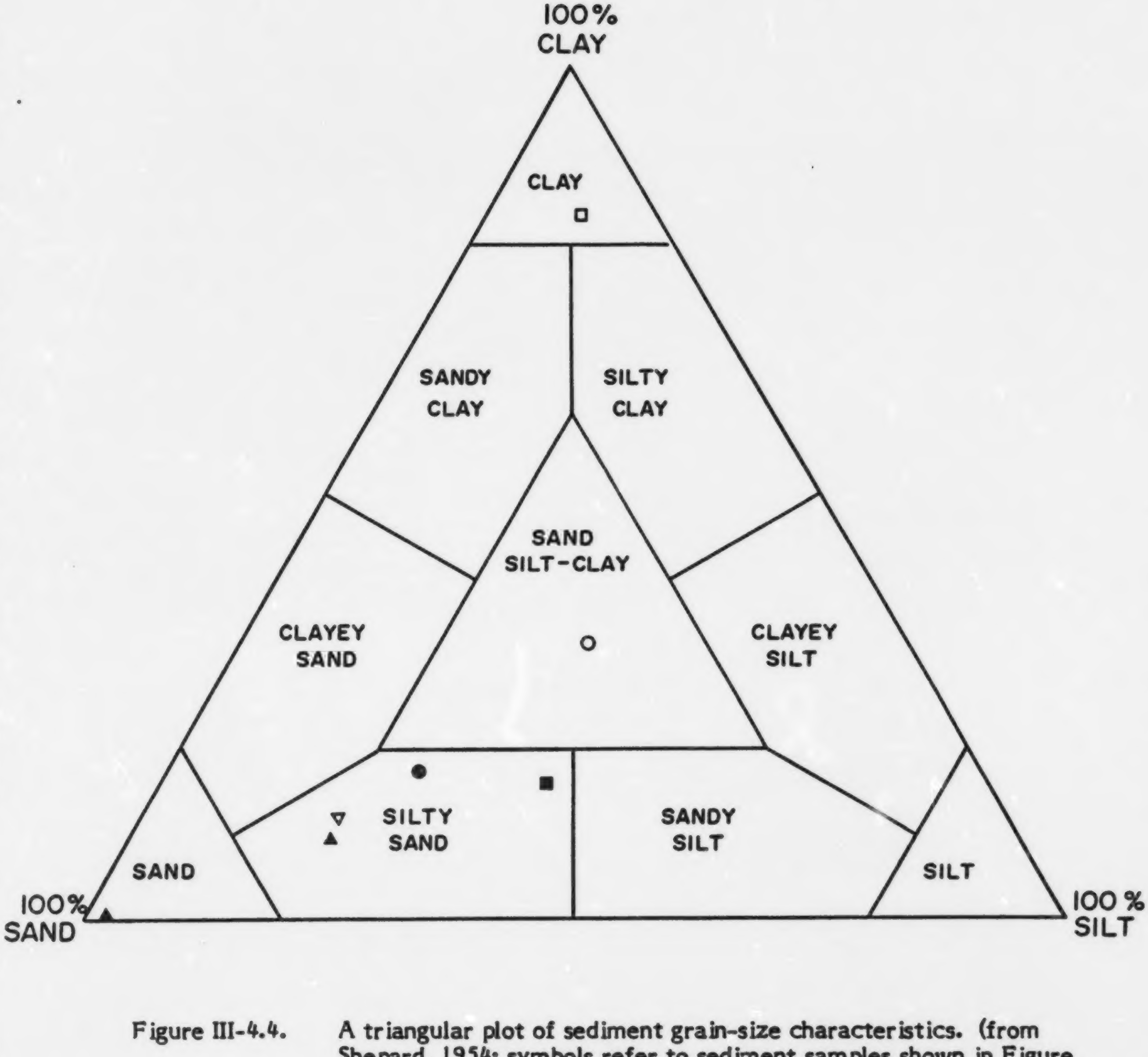

Figure III-4.4. A triangular plot of sediment grain-size characteristics. (from Shepard, 1954; symbols refer to sediment samples shown in Figure III-4.3 and given in Table III-4.5).

Methods of grain size analysis based on sieve and pipet analysis are widely accepted and remain the standard. Although slower, these methods have the advantage of modest initial equipment costs. Automated settling tube methods however, offer many advantages in clay to sand size ranges in terms of speed, sample requirements and data handling. Settling tubes for sand analysis are not commercially available and therefore are relatively expensive in terms of capital costs and set-up time. Automated settling tube methods for clay and silt are available as commercial instruments (Stockman and Fochtman, 1977). Electronic particle counting also offers very definite advantages in the clay size to fine sand size range. This is probably the only method which is field adaptable, at least for shipboard use. These instruments are commercially available but expensive and therefore not likely to be found in smaller environmental laboratories.

Sample preparation time is longer for "micro" methods and more care required to obtain a representative sample. Preparation times also increase as particle size decreases. Sample preparation will require considerably more time than the analysis by an automated settling tube or electronic counting methods.

Ultimately, all methods will provide results useful for the interpretation of environmental data. The choice will depend on the number of samples to be processed, detail required, type and amount of sediment, and precision requirements.

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5. LEAD-210 AS A MEASURE OF SEDIMENT ACCUMULATION RATES

5.1 Background

The rate at which sediment accumulates is an important consideration in evaluating depth related features in sediment cores and in predicting the impact of anthropogenic inputs relative to natural fluxes. There are a number of methods for estimating rates of sediment accumulation depending on location and sediment characteristics. Direct measurements of the downward particle flux can be made by trapping settling water column particulates (Gardner, 1980; Hargrave and Burns, 1979). Estimates can also be derived from stratigraphic inferences based on total depth of sediment in relation to the estimated time of deposition or from budget calculations relating to the particulate flux associated with river run-off and the concentration of particulates in the water column (Thomas et al., 1983). Finally analysis of sediment cores can be made based on paleontology and radiometric methods. In general, no single method is universally applicable and it is desireable to obtain estimates from two or more methods to improve reliability. The determination of geochronologies using artificial and natural radionuclides has become well established as a means for dating marine deposits over periods of time of tens to millions of years (Turekian and Cochran, 1978; Goldberg and Bruland, 1974). From an environmental perspective, recent processes are most important and this reduces the number of useful radionuclides considerably.

Several man-made radioactive nuclides such as Cs-137 and Fe-55 produced in nuclear explosions provide a useful means of dating recent deposits since the onset of major nuclear testing in the early 1950's. Cs-137 ($t_{1/2}$ = 30 yr) is the most commonly used artificial nuclide because of its stability and because it can be directly measured in core sections by non-destructive gamma counting using a Na(Ti) or Ge (Li) detector coupled to a multi channel analyzer. This allows unambiguous detection of Cs-137 gammas (Krishnaswamy et al., 1971; Robbins and Edgington, 1975). Nuclear testing and hence fallout of Cs-137 peaked in 1963. Cs-137 enters the water column via run-off and atmospheric fall-out. It has a long oceanic residence time due to its lack of reactivity in solution (it is the most electropositive and alkaline element known). A small portion, however, does reach the sediment largely via ion-exchange onto clays (Swan et al., 1982). Cs-137 and other man-made radionuclides are useful for identifying a horizon in the sediment and sedimentation rates calculated by this method will therefore be an estimate of the average sedimentation rate over the last 30 years. Man-made radionuclide profiles should show a sharp break with depth. A very diffuse profile can help to identify where mixing has taken place either as a result of sampling or as a result of infaunal mixing or from physical events such as slumping or ice scouring.

Lead-210 is the most commonly used natural radionuclide for dating recent sediments. Its chemistry and half-life permits dating for periods of 100-150 years. Pb-210 methods have been reviewed in detail by Robbins (1978) and have been applied to a variety of lake and coastal marine deposits. Dating using a natural radionuclide is not via the identification of a horizon but rather is based on the decay of the isotope after incorporation into the sediment. The following review has been limited to a discussion of Pb-210 dating although many of the references cited also describe Cs-137 dating methods.

5.2 Introduction

Lead-210 is a member of the U-238 natural radioactive series and has a half-life of about 22.3 years. There does not appear to be a significant amount of Pb-210 produced in nuclear explosions.

The U-238 decay series is shown in figure III-5.1. The usefulness of Pb-210 in dating sedimentation relates to its mode of formation and strong affinity for particulate matter. Some of its noble gas parent Rn-222, formed by decay of Ra-226 in the earth's crust diffuses into the atmosphere. Pb-210 is formed in the atmosphere via the decay of a series of short-lived precursors (Figure III-5.1). Atmospheric Pb-210 is quickly removed through precipitation (the residence time of Pb-210 in the atmosphere is on the order of days (Moore et al., 1973)). Pb-210 produced in ground water or reaching the continents is strongly adsorbed on particulates so that very little reaches the ocean. It has been shown that virtually all Pb-210 reaching the oceans is via atmospheric transport (Turekian and Cochran, 1978). In sea water, Pb-210 has a short residence time and is very efficiently scavenged by particulate material and transported to the sediment. The atmospheric derived Pb-210 delivered to the sediments is in addition to Pb-210 occurring in the sediments via in situ decay of Ra-226. This quantity of isotope termed "supported" is assumed at a steady state and forms a constant level background. The atmospherically derived Pb-210 is referred to as unsupported or excess Pb-210.

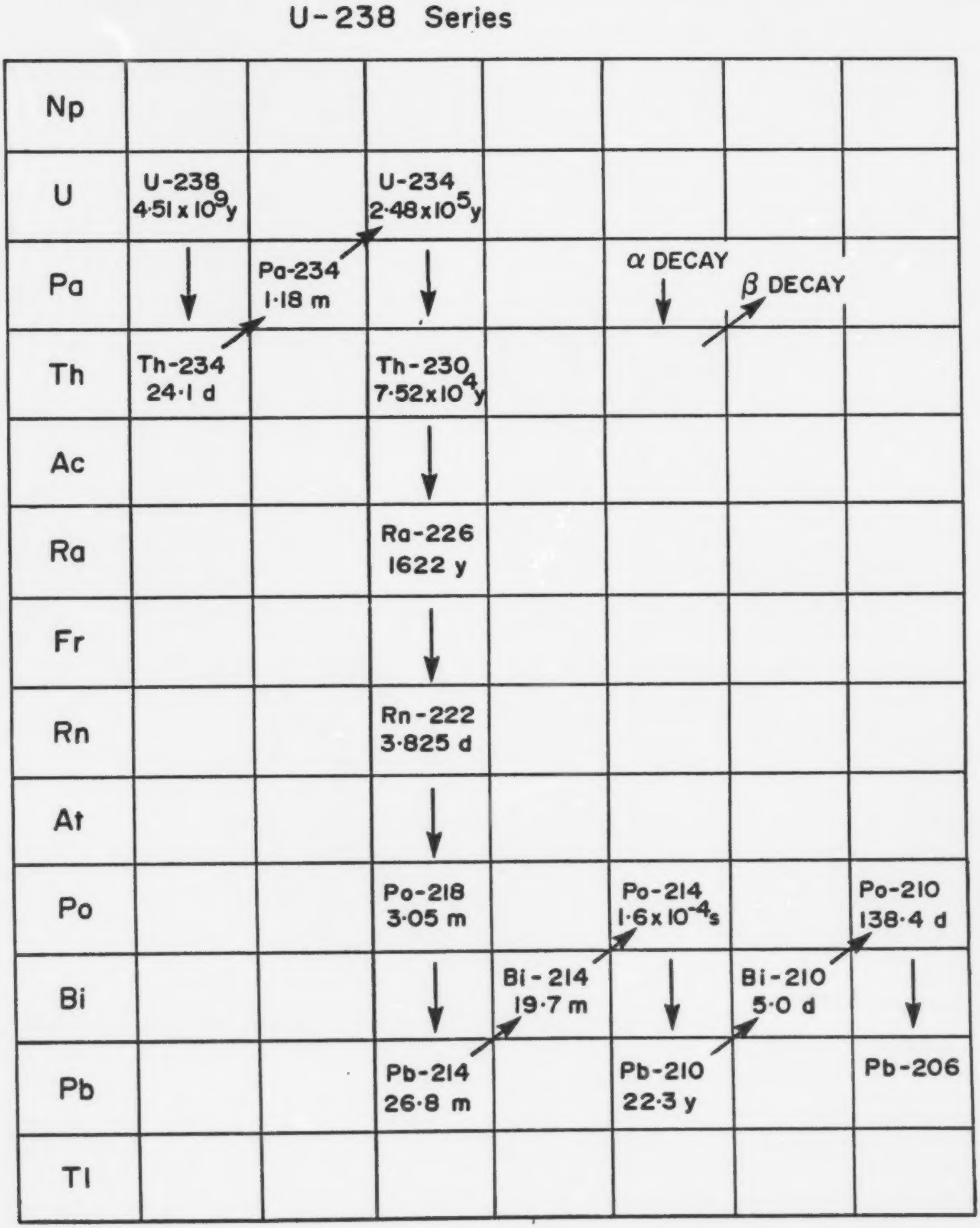
U-238 Series
Np
U
Pa
Th
Ac
Ra
Fr
Rn
At
Po
Bi
Pb
Tl
U-238 4·51 x 10^9 y
U-234 2·48 x 10^5 y
Pa-234 1·18 m
Th-234 24·1 d
Th-230 7·52 x 10^4 y
Ra-226 1622 y
Rn-222 3·825 d
Po-218 3·05 m
Po-214 1·6 x 10^-4 s
Po-210 138·4 d
Bi-214 19·7 m
Bi-210 5·0 d
Pb-214 26·8 m
Pb-210 22·3 y
Pb-206
α DECAY
β DECAY

Figure III-5.1. U-238 radioactive decay series.

Atmospheric fluxes of Pb-210 in arctic regions are lower than in more temperate regions. Naidu et al., (1981) have reported an average flux of 0.08 $dpm \cdot cm^{-2} \cdot y^{-1}$ for the Alaskan Beaufort Shelf. This is an order of magnitude lower than fluxes measured in the northeastern United States and California.

For practical purposes, the method provides estimates of time scales of up to 5 half-lives (~ 100-110 years). In ideal conditions, (i.e. undisturbed sediments) the unsupported or excess Pb-210 concentrations exhibit an exponential decay in activity with depth of burial. However, sediments are often disturbed by biological or physical processess and by variation in sedimentation rate which purturbs the expected exponential decay curve. Despite the potential problems of interpretation, the method can be extremely useful and it has enjoyed widespread acceptance for dating lake and coastal marine sediments (Krishnaswamy et al., 1971; Koide et al., 1972; Goldberg et al., 1978; Bruland, 1974; Matsumoto and Wong, 1977; Thomas et al., 1983; Schell and Nevissi, 1977).

5.3 Sampling for Pb-210

Sampling for Pb-210 dating should satisfy the criteria for all types of sediment sampling (Section II-2-1) with particular emphasis on collecting the sediment surface intact (Swan et al., 1982). The other criteria is that the core be long enough such that excess Pb-210 will have decayed away in the deeper portions of the core. Since this will be in the upper 50 cm and usually much less in arctic regions, long cores are not required and slow entry coring procedures should be used. Box corers or wide diameter cylindrical gravity corers offer the best features. Sediment should be fine grained (mainly silt and clay) as this material will have a higher Pb-210 content.

After collection, surface water should be drained by careful siphoning to minimize disturbance at the sediment/water interface and to avoid loss of "fines" and surface flocs. Some water should be saved for salinity analysis if concurrent measurements of bottom water salinity are not being made. This is required for porosity calculations.

It is most convenient to freeze the core intact in the core barrel or liner and to section later. Several authors have "quick" frozen cores using a dry ice bath immersion tank (Bruland, 1974; Swan et al., 1982). Dry ice will not normally be available for arctic sampling. Quick freezing is most important in large diameter cores (15 cm or more). "Slow" freezing will result in a partitioning of water and sediment. If sediment is reasonably compact, cores may be extruded and subsampled in the field. In either case, before subsampling, the material near the tube wall should be discarded as this will likely be contaminated with sediments from further up the core and possibly by the core barrel. Extrusion of cores is best carried out with a tight fitting piston device (see Section II-2.1; Kemp et al., 1971).

Sectioning should be carried out at as fine intervals as is practical especially near the surface . Surface "floc", if present, should be stored separately (Macdonald et al., 1983). Thin sections (0.5 - 1 cm) are desireable for the upper 20 cm. As sedimentation rates are not known beforehand, it is necessary to store all sections. Only some need be analyzed initially, with additional samples being analyzed to fill in gaps if necessary. Samples should be stored frozen in plastic bags or jars (see Section III-2). Most digests require 1-5 g of dry sediment. Additional sediment will be required for density measurements as well as other supporting information. If insufficient sediment is available, these samples can be obtained from a second core.

5.4 Analytical Methods

There have been two basic approaches to Pb-210 measurements in sediments. Neither involves the direct measurement of Pb-210 since it is a low energy β emitter (E_{max} = 61 kev). The most common approach involves the extraction and purification of Pb from the sediment via an acid leach and precipitation and counting of the decay of Bi-210 (E_{max} = 1.17 MeV), the daughter product of Pb-210 decay. The other approach used involves extracting and isolating Po from the sediment and counting the α decay of Po-210 (E_{max} = 5.3 MeV) which is assumed to be in secular equilibrium with Pb-210. Determination of either Pb-210 or Po-210 is a multi-step process. Briefly, the metal is extracted from the sediment by a hot acid leach, purified by extraction from the leachate and then collected as a precipitate. Po-210 is measured directly, while Pb-210 is allowed to equilibriate with Bi-210. The various methods that have been employed are summarized in Table III-5.1.

Quantitative recoveries are not required but consistency is, as the data are used to determine relative changes (rates) of nuclide with depth in the sediment. Therefore, all methods must employ some means of estimating the yield of each digest and separation. A carrier is usually added and used as the basis for estimating yield. Evaluation of the methods is difficult as there have been no intercomparisons made. All methods seem to satisfy the precision criteria although efficiencies of the methods are quite variable. The methods of Koide et al., (1972) and Matsumoto and Wong (1977) have been applied to arctic cores. Cores from the Beaufort Sea shelf have been dated using the latter method (Institute of Ocean Sciences, unpublished data) while cores from Strathcona Sound, northern Baffin Island have been dated

TABLE III-5.1

SUMMARY OF ANALYTICAL METHODS FOR Pb-210 DATING

REFERENCE	QUANTITY OF SEDIMENT REQUIRED	DIGESTION	SEPARATION AND PURIFICATION OF Pb	RECOVERY/PRECISION	COUNTING
Koide et al., 1972 Krishnaswamy et al., 1971; Rama et al., 1961	2 - 10 g	initial heating at 400°C for 2-3 hours to remove organics. Residue leached in 6 N HCL for 1 hour at 100°C. Leachate dried and dissolved in 1.5 N HCL	30 mg Pb^{2+} (Pb-210 free Pb NO_3 solution) added as carrier: solution passed through anion exchange column: Pb eluted with 1.5 N HCL: Pb is precipitated as Pb SO_4 from pH2 solution.	not stated	made after allowing 20 - 40 days for in growth of Bi-210 daughter in flow-type beta counter.
Matsumoto and Wong, 1977. Macdonald et al., 1983	~3 g	20 mL conc. HNO_3 at 120°C for 2 hours: leachate diluted to 3 N HNO_3	20 mg Pb^{2+} (Pb-210 free Pb NO_3 solution) added as carrier: Pb separated by electrodeposition on a Pt anode as $Pb0_2$. $Pb0_2$ dissolved in 3N HNO_3 containing a few drops of H_2O_2 Pb precipitated as Pb S04 from pH 2 solution.	70 - 95% recovery of Pb	made after allowing 30 days for in growth of Bi-210 daughter in flow-type beta counter.
Robbins and Edgington, 1975 Cliffton and Hamilton, 1979 Flynn, 1968	up to 5 g	HNO_3 followed by several aliquots of conc. HCL. HCL treatment continued until no NO_2 fumes evolved. Solution diluted	- measurement of Po-210 - assumed to be in secular equilibrium with Pb-210. Filtrate is evaporated to small volume and heated with conc. HCL to eliminate any remaining HNO_3. Po-210 self plated onto silver planchettes with 10 mg of a bismuth carrier at 85-90°C in the presence of sodium citrate at pH2. plating times 60 min for 79% recovery.	quantitative recovery of Po-210	Po-210 alpha-activity counting done for 10^3 min.
Nriagu et al., 1979	1 - 3 g	15 mL aqua regia/g sediment heated at 90°C for 6 hours with stirring	plating times 4 hours with shaking in presence of ascorbic acid (1 g/3 g sediment).		NMC 2 proportional counter
Farmer, 1978	1 g	100 mL 8 m HNO_3 for 4 hours at 70°C	Pb-208 added during digestion to monitor yield.		
Joshi and Durham, 1976	1 - 3 g	initial heating to 450°C to destroy organics: residue subjected to successive HNO_3 HF leaches to remove silica; then 3 times with HNO_3 (2.5 mL/g). Residue fused with $NaOH:Na_2C0_3$ (3:1) at 450°C for 1 hour.	residue dissolved in 90% HNO_3 - diluted to 70-75% HNO_3 - ION EXCHANGE (Dowex 1-x8) in 1.5 M HCL. Pb eluted with 9 M HCL: precipitated as Pb $Cr0_4$.	35 - 40%	Bi-210 counted

using both methods (Thomas et al., 1983). The latter study found the results obtained by the Koide et al. (1972) method to be about 30% higher. Relative concentrations were similar, however, for both methods. The simpler methods, such as by Koide et al. (1972) are likely preferable despite lower yields. The more complex method outlined by Joshi and Durham (1976) seems to suffer from a low yield which may be a problem where excess Pb-210 levels are low. The final choice of method will be dictated by the available facilities and counting equipment. Methods which achieve high yields are preferable for arctic sediments.

5.5 Data Interpretation

There have been three basic models developed for interpreting Pb-210 depth profiles and estimating accumulation rates (Appleby and Oldfield, 1983); 1) constant flux, constant sedimentation rate (CF:CS) model; 2) constant rate of supply (CRS) model and 3) constant initial concentration (CIC) model. The models and their application and limitations are described briefly below.

5.5.1 CF:CS Model

Most applications of Pb-210 dating have utilized this model with minor refinements (Krishnaswamy et al., 1971; Robbins and Edgington, 1975; Cliffton and Hamilton, 1979; Matsumoto and Wong, 1977). The model is based on the following assumptions:

1) the flux of excess Pb-210 to the sediments is constant
2) the sedimentation rate is constant
3) there is no post-depositional migration of Pb-210 within the sediment
4) the supported Pb-210 activity is constant with depth

Under these conditions, the activity of Pb-210 in a sediment section of age t (years) will be:

$$A(t) = (F/w)e^{-\lambda t} + A' \qquad (1)$$

where F is flux of Pb-210 in $\mathrm{dpm \cdot cm^{-2} \cdot y^{-1}}$

w is sedimentation rate in $\mathrm{g \cdot cm^{-2}\, y^{-1}}$

λ is decay constant for Pb-210 = $0.0311\ \mathrm{y^{-1}}$

A' is activity of supported Pb-210 $\mathrm{dpm.g^{-1}}$ dry weight

Age (t) can be related to sediment depth (z) by the sedimentation rate S in $cm \cdot y^{-1}$, where $t = z/S$; S is related to w by sediment density, ρ_s and porosity ϕ, by the expression $w = S\rho_s(1-\phi)$.

Equation (1) can therefore be rewritten in terms of depth to give:

$$A(z) = \frac{F}{S\rho_s(1-\phi)} e^{-\lambda/S} + A'$$

A plot of ln (A(z) - A') vs z will be linear and give a slope of $\frac{-\lambda}{S}$ and intercept of $\frac{F}{w}$. Robbins and Edgington (1975) refined the model to take into account the compaction of the sediment as it accumulates. Compaction will result in a non-linear relationship between time of deposition and sediment depth. To account for this, a corrected depth z' can be calculated by correcting porosity (which decreases with compaction) to that at the surface. The relationship between z' and porosity is given by:

$$z' = z + \int_o^z \frac{\phi_o - \phi}{1 - \phi_o} dz$$

where ϕ_o is porosity at $z = o$ and ϕ is porosity at depth z.

5.5.2 Constant Rate of Supply (CRS) Model

This model described in detail by Appleby and Oldfield (1978), differs from the previous one in that the sedimentation rate is not assumed constant. However, the flux F is assumed constant. Under these conditions, $C_o(t) \cdot w(t) = \text{constant}$, where $C_o(t)$ is the initial concentration of excess Pb-210 in sediment of t years and w (t) is the sedimentation rate in $g \cdot cm^{-2} \cdot y^{-1}$. At a depth z, the age (t) can be expressed as:

$$t = \frac{1}{\lambda} \ln \frac{A(o)}{A(z)}$$

where A (o) is the total residual excess Pb-210 below depth z ; A (z) is related to C (z) by the relation:

$$A(z) = \int_z^\infty \rho(z) \; C(z) \, dz$$

The rate of sedimentation r at depth z is then given by:

$$r(z) = \lambda \frac{A\ (z)}{C\ (z)}$$

5.5.3 Constant Initial Concentration (CIC) Model

This model was also developed for areas with varying accumulation rates. The assumption is that the flux P will vary with the accumulation rate, such that sediments will have the same initial concentration regardless of changes in sedimentation rate ($C_o(t)$ = constant). Under these conditions the age t of a sediment layer (z) can be expressed as:

$$t = \frac{1}{\lambda} \ln \frac{C(o)}{C(z)}$$

where C(o) is the unsupported Pb-210 concentration at the sediment water interface and C(z) the concentration at depth z (Pennington et al., 1976).

5.5.4 Summary

No single model is applicable in all cases, while in some instances none of the models may be suitable. Appleby and Oldfield (1983) have outlined a tentative procedure for choosing which model to apply:

1. Linear profiles (ln A vs z): all models give the same chronology

2. Non-linear profiles
   a) if the total cumulative excess Pb-210 (residuals) in a core is comparable with known atmospheric flux or with levels in nearby cores, then the CRS model is likely applicable.
   b) if Pb-210 residuals vary from different sites and are in proportion to sediment accumulation rates, then the CIC model is most likely applicable.
   c) if neither case (a or b) is satisfied, dating cannot be reliably carried out.

Although the CRS and CIC models make one less assumption than the CF:CS model, they require more detailed sampling intervals and are subject to false interpretation. Physical and infaunal mixing as well as episodic events such as slumping or ice scouring would result in non-linear (ln A vs z) plots and could be interpreted as changes in sedimentation rate or flux. For these reasons, the dating of sediment cores must be done with caution and supporting data sought from independent dating methods and other measurements which might indicate whether or not the sediment has been mixed or disturbed. Swan et al. (1982) have noted that supported Pb-210 may not be constant with depth and that Ra-226 measurements should be made to compensate for this.

Other supporting measurements can include porosity which is required to correct for compaction, organic carbon content, grain size and visual features such as colour changes or laminae. Deviations or discontinuities in the sediment depth profiles of these or other measurements can identify alterations in deposition patterns and aid in resolving deviations in the profiles of supported and/or excess Pb-210. This auxiliary information is particularly important where the surface sediments have become well mixed as Pb-210 levels may still decrease with depth.

In much of the Arctic, sedimentation rates will be low (usually less than 1 $mm.y^{-1}$) and the Pb-210 flux low because of the low levels of precipitation in most areas and the presence of ice cover for many months of the year. Excess atmospheric Pb-210 fallout may be trapped in multi year ice or transported from the area of deposition before being released. For these reasons, Pb-210 dating may not be practical in many regions. Lead-210 dating in the Canadian and Alaskan Beaufort Sea (Ocean Chemistry Division, IOS, unpublished data; Naidu et al., 1981)) and in Strathcona Sound, northern Baffin Island, (Thomas et al., 1983) have found low Pb-210 activities; excess Pb-210 had decayed to zero at a depth of 5-10 cm in Strathcona Sound.

Lead-210 determinations are carried out by only a few commercial testing laboratories in Canada. Costs for analyses excluding sample preparation will be in the range of $50-75/sample for analysis by a method similar to that of Koide et al., (1972).

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6. ANALYSIS AND PROCESSING OF ZOOBENTHOS SAMPLES

6.1 Introduction

Methods for the treatment of zoobenthos samples have been presented by numerous authors (e.g., Thorson, 1957a; Birkett and McIntyre, 1971; Stirn, 1981; Eleftheriou and Holme, 1984). The following review will consider the treatment of macrofauna samples with special reference to arctic sampling.

A benthos sample collected by grab consists of bulk sediment which must be processed to remove the organisms. Macrofauna samples may range in volume from a few litres to 50 or more; several replicate samples for separate analysis are usually collected at each station and a group of stations is often sampled on a single day. The process of extracting organisms is generally divided into an initial concentrating stage (done in the field) and a sorting stage (often performed in the laboratory). Time and manpower constraints and limited laboratory facilities in the Arctic generally dictate that this approach be used. The specific objectives of the sampling program, however, as well as the resources available, should determine the amount and type of sample processing to be done in the field (see Section 6.2.1.1).

There are several advantages to processing and rough sorting live benthos samples in the field:

a) Live specimens are easier to extract, sort and identify.

b) Live specimens of biomass-dominant groups (e.g., polychaetes, molluscs, crustaceans) can be weighed fresh to give the most representative estimate of wet biomass (Howmiller, 1972; Stirn, 1981). Storage in preservatives such as formalin, ethanol and isopropanol may result in significant changes to wet weight and dry weight (Thorson, 1957a; Stirn, 1981) and will also change length-weight relationships. An alternate technique is discussed in Section 6.2.1.1.

c) Some organisms contract in fixation or preservation fluids and entirely change their natural shapes (e.g., actiniarian and other "soft" cnidarians, opistobranchian and other molluscs lacking an exoskeleton, nemertine worms, echiurids, priapulids, sipunculids and some polychaete worms). If these groups are transferred alive into sea water to which a narcotising agent (e.g.,

$MgCl_2$, 4%; menthol crystals, or propylene phenoxetol, 0.15%; (McKay and Hartzband, 1970; Stirn, 1981) is added, the organisms usually become fully expanded and relaxed after a period of 10-15 minutes. They can then be transferred into a fixative where they no longer contract due to anaesthetisation. This process minimises damage to delicate specimens and greatly aids identification (McKay and Hartzband, 1970). The suitability of a particular procedure for anaesthetisation of specimens should be checked during preliminary sampling.

d) Damage is reduced to animals with delicate body structures, such as those listed above, if they are separated from the bulk sample and preserved in separate jars during storage and transportation (Stirn, 1981).

6.1.1 Procedures for Processing Zoobenthos Samples

Based on relevant references (e.g., Birkett and McIntyre, 1971; Stirn, 1981; TetraTech, 1982; Eleftheriou and Holme, 1984) and practical experience, the following procedures for handling benthos samples are suggested:

a) The total volume of each sediment sample should be measured in a calibrated container (e.g., Lie and Pamatmat, 1965; Christie, 1975).

b) Each replicate sample should be separately sieved, and fixed in the field on the day of collection (as soon after sample collection as possible).

c) Each replicate sample should be separately sorted and enumerated for species composition and abundance to determine variability within the station series.

d) The extraction of macrofauna should be done (at a minimum) by sieving the whole sample on a sieving table or hopper with a 0.5 mm mesh sieve with the aid of a sea water sprinkler (e.g. Holme, 1959). It is suggested that a series of mesh sizes, 0.5, 1.0 and 10 mm, be used in sieving if possible since the 0.5 mm sieve retains elongated specimens of macrofauna which may pass a 1.0 mm sieve. A 10 mm (or other coarse mesh size) screen separates coarse mineral or detrital particles and large benthos specimens from the finer fractions. The large specimens should be preserved in separate jars to prevent damage to delicate infauna (e.g., Section 6.1). The separation and sorting of size fractionated samples is also easier and more convenient. The minimum sieve size selected will depend on the purpose of the survey (Birkett and McIntyre, 1971; Eleftheriou and Holme, 1984) but for quantitative surveys, use of a 0.5 mm screen should be standard for macrobenthos. To obtain maximum information, the different size fractions should be sorted and identified separately.

e) All organisms and other residue on the screens should be quantitatively transferred into a suitable jar. Sturdy plastic jars and lids (e.g., polypropylene wide-mouth jars and closures) are more resilient in transport than glass jars. The organisms should be fixed in a buffered solution of 5-10% formalin in sea water following narcotisation where appropriate (Section 6.1). (Note that 100% formalin is the undiluted formaldehyde (40% concentration) solution.) Borax, hexamine or marble chips may be used to buffer the formalin solution against acidification. After an initial fixation period (1 week to 1 month, but preferably 1 week) the specimens should be washed free of fixative and transferred to 70% ethanol or isopropanol to prevent decalcification of delicate calcareous structures and to reduce the exposure of taxonomists to formaldehyde fumes. Vital staining techniques (e.g., with Rose Bengal) may be used at the time of initial fixation to aid in sorting (Holme and McIntyre, 1971; 1984; Williams and Williams, 1974).

Note: Formaldehyde, ethanol and isopropanol are restricted items for air transport. Jars containing these solutions should be sealed with vinyl tape and double-bagged in plastic to prevent leakage of contents. Consult the latest IATA regulations for shipping restrictions (Section II-8).

f) Permanent labels made of paper with a high rag content bearing all of the pertinent sampling information should accompany each sample (and subsample) throughout all stages of processing, transportation and storage. Although it is useful to write the station number, date, etc., on jar lids and sides with a waterproof pen, it is essential to place a strong waterproof label inside the jar as soon as the sample is sieved or sorted. The labelling material (waterproof ink) should be inert if other analysis are to be done on the samples. The labels should include:

i) sampling agency or organisation
ii) cruise or program number
iii) research vessel (or vehicle)
iv) sampling station: number, location, depth, replicate number
v) date and time of collection
vi) gear used and area sampled
vii) volume of sample
viii) mesh size(s) of sieves (nets, etc.)
ix) surname(s) of collector(s)

In addition to the label information, sampling log sheets should be used to record detailed remarks, available information on sampling conditions (e.g. sea state, substrate type, ecological data) and other types of observations or samples which were taken at the same site (e.g., photography, video, salinity, temperature, particle size, chemical parameters, etc.). This information should be retained in permanent files to be subsequently combined with the data on species composition, abundance, particle size distributions and physico-chemical data. The suggestions on labelling and log sheets also refer to samples of phytobenthos (Section II-5).

Detailed descriptions of variations on procedures for handling benthos samples (sieving, elutriation, differential flotation and physico-chemical extraction) are provided by Birkett and McIntyre (1971), Barnett (1980), Stirn (1981), Eleftheriou and Holme (1984) and references cited therein. Elutriation is a process in which a continuous upward stream of water is passed through the sample in a container with an overflow to a fine collecting sieve. The flow rate is regulated so that the water agitates the sample and elevates the small animals but not the sediment particles (e.g., Sanders et al., 1965). Several devices which have been used successfully for macrofauna use both water and air jets (e.g., Lauff et al., 1961; Worswick and Barbour, 1974).

Differential flotation uses the differences in specific weight between the organisms and the sediment in combination with a medium of a suitably high density so that the animals float clear of the heavier debris. A variety of liquid media have been used with varying success, including carbon tetrachloride, sugar solutions and $ZnCl_2$ solution. A principal disadvantage is that organic detritus also floats making the techniques unsuitable for muds and silts without adjustment of the specific gravity of the flotation medium. Other disadvantages are that most of these methods are messy, and with carbon tetrachloride, potentially dangerous unless precautions are taken to avoid inhalation of the vapour (Dillon, 1964). Carbon tetrachloride liquid should always be covered with water and the process should be carried out under good ventilation. An alternate flotation medium is the colloidal silica polymer, Ludox^{TM}, which has been used to separate nematodes from sediment and detritus (De Jonge and Bouwman, 1977) and for the successful separation of macrofauna species (Eleftheriou and Holme, 1984).

A method of physico-chemical extraction of fauna from fine clays and compacted silt involving treatment of the bulk sample with formalin followed by freezing and thawing was described by Barnett (1980). Petersen (1977) described procedures for handling benthos samples collected through the ice in winter. The applicability of the above methods for arctic benthos sampling will depend on the particular purpose of the sampling and the availability of facilities and manpower.

6.1.2 Sorting of Benthos Samples

Errors arising from sorting of samples are one of the major sources of error influencing the inferences which benthic ecologists derive from a benthos survey (Barnett, 1979; Coleman, 1980). The other major sources of error, inadequacies in survey planning and deficiencies in methods of sampling, have received considerable attention in the literature as indicated in other sections of this review. The subject of sorting errors, however, has not been widely discussed. Sorting errors can be systematic or random and since true numbers of organisms in samples are not known, the magnitude of sorting errors is difficult to assess. Coleman (1980) has considered the problem of sorting error and its significance in interpretation of results of benthos surveys and to planning of survey strategy. Sources of sorting error cited by Coleman (1980) are:

1) difficulty of separating small organisms from samples with large amounts of vegetation, detritus, or bulky colonial organisms (e.g. sponges, bryozoams, hydroids, mussels, etc.) with numerous nooks and crannies which might conceal small fauna.

2) for molluscs, the difficulty of determining whether small shells contain an animal or not.

3) boredom and inattention on the part of sorters depending on experience, work habits and length of time spent sorting on a particular day.

4) limited time and labour for completion of task (accuracy may be sacrified for speed in sorting).

5) choice of mesh size for screening samples and thus, size of specimens: the greater the proportion of small organisms retained in the screened sample, the greater may be the chances of inaccuracies in sorting.

There are a number of ways to minimize sorting errors, although it is doubtful if they can be eliminated altogether. The use of low-power stereoscopic microscopes and transluscent channelled counting trays with background grids (e.g., Schlieper, 1972) greatly increases the thoroughness of finding small organisms in benthos samples. Separation of bulky specimens with complex surfaces at the time of collection or sieving may eliminate some of the difficulties in subsequent sorting of the smaller organisms (see also Section 6.1.1(d)). Large objects removed from benthos samples should be inspected for small organisms on their surfaces.

The use of bulk vital-staining (e.g., Rose Bengal) makes the animals easier to see. Small molluscs may be checked for viability at the time of collection if, after identifying the specimens from the shells, they are crushed and examined for the presence of mollusc tissue (verification of identity is impossible, however, if this technique is used).

The efficiency of sorting may be checked by quality control procedures (Section 6.2.3), and by examining the distribution of individuals among the species present. Deviations from a log-normal distribution may reflect environmental conditions, but may also reflect sorting error. The failure to find rare species or the inaccurate identification of species can produce deviations from the log-normal distribution (Gray and Mizra, 1979).

The principal consequences of sorting error are the underestimation of population density and number of species present. Coleman (1980) examined the results from re-sorting of benthos samples by workers who were not told they were repeating the step. Some of the samples were given to the people who had originally sorted them, others were given to different assistants. He found that re-sorting a single grab sample increased the number of species found by an average of 27%. On the basis of species per station, the number of species rose by 13% when a single grab was re-sorted. When all three grabs at a station were re-sorted, the increase in number of species per station was still about 13%. The number of individuals (population density) per grab sample increased by about 37% after re-sorting. When all three grabs per station were re-sorted, the increase in population density per station was of a similar level. Polychaetes and crustaceans which together accounted for over 80% of the species and individuals were the taxonomic groups which were most affected by the re-sorting, whereas molluscs, the third most abundant group samples were only underestimated by about 5%.

The significance of sorting error, in general, will depend on the objectives of the study. If absolute values for population density are necessary, then the underestimation of individuals may be a significant factor. If the objective is to compare faunal densities over areas or time, then the underestimation may not be as serious, assuming that the sorting error is the same for all samples. Use of the same sorters for each set of samples will help to maintain the consistency of sorting. If the aim is to compare stations on the basis of species composition, then the effect of sorting error is greatest when single samples are compared but lessens as more grabs are taken at a station.

6.2 Analysis of Zoobenthos Samples

6.2.1 Estimation of Biomass

Biomass, or standing crop, is defined as the amount of living substance constituting the population of organisms which are being studied (Crisp, 1971). It normally refers to the weight of a sample population of a species or group of species which inhabited a sampled surface area or volume. Biomass of benthos is generally reported on a m^2 basis for comparibility and convenience, and is calculated from the mean of replicate samples from a single station or group of stations.

Biomass can be expressed in units of:

a) wet weight;
b) whole or decalcified dry weight;
c) ash-free dry weight (weight of organic matter);
d) carbon and/or nitrogen content;
e) caloric or energy equivalent.

6.2.1.1 Wet Weight

Wet weight is the least reliable measurement of biomass. It is best performed on unpreserved specimens since preservation generally results in a significant loss of organic material by dissolution (e.g. Thorson, 1957a; Howmiller, 1972). Wet weighing can be done in the field on a top-load balance unless samples are so small that an analytical balance is needed. Before being weighed, specimens should be cleaned of sediment and detritus, and blotted to remove surface moisture. (In the laboratory, an alternative method for removing externally adhering water from small organisms is to place them on screens in modified 50 mL cellulose nitrate centrifuge tubes and to spin them for one minute; see Howmiller, 1972, for details.) Removal of shells or other hard parts from large specimens of molluscs or polychaetes is not recommended if it cannot also be done to smaller species or individuals of the same taxonomic groups. For some large organisms it is not feasible to use this treatment (e.g., calcareous algae, madreporaria, bryozoans). If this approach is used, however, it should be clearly stated.

An alternative means of estimating wet biomass if specimens cannot be weighed in the field is to collect a parallel series of samples which can be deep-frozen and used for wet (and dry) biomass determinations in the laboratory (Stirn, 1981).

Wet biomass estimations have frequently been reported in the literature based on preserved wet weights of organisms. These data have little value unless the preservatives used and the duration of preservation before weighing are described (Howmiller, 1972). Since wet weights (and dry weights) tend to reach a relatively constant value after about a month, it is possible to determine factors for back calculation to live wet weight or true dry weight. Because the factors would likely depend on variables such as the species and condition of specimens, the chemical composition of the preservative and so on, the circumstances for valid biomass estimation would have to be similar to those for determination of the factors (and vice-versa). It is recommended, therefore, that quantitative studies of the benthos include efforts to estimate the possibly significant weight losses which occur with the particular methods used (Howmiller, 1972).

6.2.1.2 Dry Weight

The weight of completely dehydrated specimens is the dry weight or biomass. There are three major methods for removing water:

1) heating in an oven at 70-100°C, atmospheric pressure;
2) drying under vacuum at temperatures up to 60°C;
3) freeze drying or lyophilization.

All three methods give similar results provided that enough time is allowed for complete dehydration. Since larger organisms clearly require longer to dry to constant weight, a test should always be run to check the minimum time for complete drying before a large series of determinations is started with a given drying apparatus. If an oven is used, temperatures should not exceed 100°C, since there will be losses of volatile tissue substances. If the temperature rises to 110°C during drying a loss of volatile soft tissue components of up to 10% by weight in 48 hours may occur (Giese, 1967). On the other hand, gentle drying at room temperature is much slower and tissues may autolyse. A good compromise is to dry under vacuum at 60°C, a temperature which denatures most enzymes but does not result in serious volatilization losses. Tissues dried by this method are also usually suitable for calorimetry or dry-ashing (Crisp, 1971, 1984). Freeze drying is generally the gentlest method and is recommended if further chemical analyses are to be performed on the samples. Dried samples are best stored sealed in a deep freeze or under continuous dessication at room temperature.

In order to avoid the time-consuming step of transferring samples to other types of dishes for the ashing procedure, the same dishes (silicon or heat resistant crucibles) should be used for both procedures. Top-loading balances may be used for large samples and analytical balances (with ±1% reproducibility) for small macrobenthos samples.

Specimens for dry biomass determinations must be clean of sediment or detritus and completely free of sea water. They should therefore be rinsed for a period (up to 30 minutes) in distilled water prior to drying (Stirn, 1981). Calcareous exoskeltons may be removed mechanically or through decalcification treatment (e.g. Humason, 1965, p. 29-31) if applied consistently to all groups. Treatment with the Formic Acid A decalcification solution of Humason (1965) described below is effective and relatively rapid, especially if the higher level of formic acid content is used.

Formic Acid A

formic acid	5.0 - 25.0 mL
Formalin, concentrated	5.0 mL
distilled water to make	100.0mL

With 25 mL formic acid content, small specimens (e.g., juvenile molluscs) will be decalcified in about one hour. The small specimens should be closely monitored when in this concentrated solution to prevent tissue losses due to over exposure to the acidic medium. Large specimens of animals such as gastropods and bivalves may need up to 3 days for decalification.

Biomass results which are more accurate than those attainable through decalcification may be obtained, however, by the ashing procedure (Section 6.2.1.3). If seasonal surveys are carried out in a given area, it is essential that every drying operation is conducted under identical conditions. This prevents variations in drying efficiency which may be falsely interpretted as a seasonal effect such as a change in condition factor (Crisp, 1971, 1984).

6.2.1.3 Ash-free Dry Weight

The ash content of the benthos, consisting of body substances such as inorganic salts, silica, calcium phosphate and carbonate, is estimated after dry weighing by incinerating the organic substances in a muffle furnace. To ensure complete burning of all organic matter, a period of four to six hours at 500-600°C is suggested (Crisp, 1971, 1984). It is desirable to use equipment with stable thermoregulation and a thermorecorder (Stirn, 1981). When incineration is completed, the samples are cooled to about 80°C in the open, and then to room temperature in a desiccator, before weighing on an analytical balance or microbalance. Ash-free dry weight which is calculated by subtracting the ash weight from dry weight, is a reliable estimate of total organic matter for samples consisting manly of soft-bodied organisms. For samples with a high proportion of calcareous exoskeletons, the ash content is seriously underestimated by this method since there may be a significant volatilisation of inorganic carbon dioxide from carbonates and hydrated water from silicates (Crisp, 1971, 1984). For samples with appreciable numbers of molluscs, barnacles, echinoderms and so on, these groups should be treated differently, so that the dry organic weight of the soft tissues and exoskeleton can be estimated separately. Crisp (1971, 1984) has reviewed appropriate techniques for the various groups involved.

An alternative method of biomass estimation in units of mass of living tissue only is to chemically analyse the organic tissue for nitrogen content, which is assumed to be proportional to the amount of organic matter. This approach, therefore, avoids dealing with the problem of non-living parts of the organisms with which the previous methods were contending. Giese (1967) has reviewed methods of biomass estimation based on nitrogen analysis. Details of the classical Kjeldahl measurement of nitrogen are given by Barnes (1959; pp. 148-150). Because the results of a nitrogen analysis vary slightly with the technique used, the procedure should be stated and indicated in the unit, e.g., biomass (mg Kjeldahl nitrogen).

6.2.1.4 Measurement of Caloric Content

This is the measurement of biomass as the amount of energy released from the tissues when they are fully combusted to water and carbon dioxide. This approach overcomes the problem of defining what are the "living" and "non-living" parts of the organisms because generally there is no release of energy when non-living components (e.g., water, inorganic substances) are combusted. In addition, energy units have exactly the same meaning for all organisms, and energy transfers between trophic levels can be accounted for. When biomass is measured in energy units, the direct way is to determine the caloric content or the heat of combustion per gram dry weight of the tissue in a bomb calorimeter. Phillipson (1964) has provided detailed methods for the micro-bomb calorimeter which analyses samples of 5 to 10 mg. Crisp (1971, 1984) has reviewed calorimetry and indirect chemical methods of measuring the caloric content of organic matter. Hughes (1969) described an iodate oxidation method of determining the total amount of oxidisable organic matter which avoids most of the errors associated with wet oxidation by dichromate (Crisp, 1971, 1984). Results from the iodate oxidation method of Hughes (1969) are consistent with those obtained by direct calorimetry.

6.2.2 Taxonomic Identification and Enumeration

6.2.2.1 Introduction

The analysis of benthic communities is based on accurate identification and enumeration of specimens from benthos samples. Since there are usually many major and minor taxomonic groups represented in the benthos from most marine and estuarine areas, a comprehensive study of benthos requires a team of skilled taxonomists for the identification of the most important classes, and access to outside expertise for assistance in identification of remaining biota. It is suggested that a study team include taxonomist specialists for polychaetes, crustaceans (e.g., amphipods, isopods and cumaceans), molluscs and echinoderms. Non-specialist members can identify the taxonomic groups which are usually represented by a small number of easily recognisable species (e.g., sipunculids, cnidarians, etc.), provided that the fauna of the region have been studied previously and resource materials are available (Stirn, 1981). Consultant specialists may have to be found for the identification of sponges, bryozoans and ascidians. Mayr (1969) and Gosner (1971) have discussed the types of taxonomic keys and have provided informative introductions to the practices of systematics and identification.

Specimens of the major taxonomic groups may be identified at the species level or temporarily coded in cases where a species is clearly recognizable but the positive identification needs further work. The abundance of all species present in the sample is counted and recorded either during the identification process or afterwards. Only specimens which were viable at the time of collection are included, not skeletal or other remains. When damaged specimens are encountered, only the heads are counted as specimens, not appendages or other body parts (Stirn, 1981).

The services of public natural history museums can be valuable for information, confirmation of identifications and deposition of representative specimen. Contacts in the National Museum of Natural Sciences, Ottawa, Ontario, K1A 0M8, are listed in The Directory of Marine and Freshwater Scientists in Canada; (Anon., 1984).

6.2.2.2 References Applicable to Identification of Arctic Benthos

A list of references which will be useful for the identification of arctic benthos is provided in Appendix D.

6.2.3 Quality Assurance in Benthos Studies

The purpose of a quality assurance program for a measurement system is to reduce errors to tolerable limits and to ensure that the measurements are likely to be of acceptable quality (Taylor, 1981). Quality control is the mechanism within a quality assurance program which controls errors, while quality assessment is the means to verify that the system is operating within acceptable limits. Since quality is subjective and varies according to situation, tolerable limits of error must be determined on the basis of cost-benefit considerations for each property to be measured. Quality control in benthos studies is complicated by the fact that true values of parameters such as species density and biomass for natural populations are unknown. Therefore, it is impossible to determine the "accuracy" of the methods used (i.e. how close the estimates are to the true values). Precision (the relative difference between replicate or repetitive measurements) of benthos estimates can be established, however.

Measures for quality control which are applicable to benthos studies (e.g. Stirn, 1981; Tetra Tech, 1982; MacGregor, 1983) include:

1) Good laboratory practice, which requires that:
    a) proper procedural protocols are written and observed in practice. These include sampling protocol, steps for quality control in the field, methods of sample treatment, methods of analysis, quality control steps in the laboratory and standardized calculation and data reporting;
    b) high standards of personnel training and laboratory management are used; and
    c) field and laboratory procedures are inspected and audited by a third party.

2) Field sampling quality control including:
    a) collection of an adequate number of samples;
    b) replication to the extent possible of a fixed percentage of samples;
    c) preparation of detailed written sampling directions;
    d) documentation of sampling in field log books;
    e) verification of the number, type and location of samples obtained;
    f) careful selection, use and maintenance of sampling equipment;
    g) adequate inspections to guard against inadequate sample preservation, leakage or loss during collection, transfer, shipment and storage;
    h) adequate chain of custody and labelling for samples; and
    i) collection of reference (background) and contingency samples.

3. Laboratory quality control:

   a) preparation of detailed written protocols for sorting and identification;

   b) a fixed percentage (e.g. 5%) of all samples should be resorted by individuals different from those who conducted the original sorting. This should be a double-blind test. Results of the second sorting should be maintained.

   c) a fixed percentage (e.g. 5%) of the species identified by each member of the taxonomic team should be verified by a competent independent taxonomist. Results of the verifications should be maintained.

   d) complete records or log sheets of sorting, processing and laboratory analysis of each replicate sample should be maintained in a file with sampling data (Section 6.1.1 (e)).

   e) a voucher or laboratory museum collection of specimens which are representative of each species (or lowest taxon) collected during benthos surveys in a study area should be prepared and maintained. Efforts should be made to have the identifications verified and representative specimens deposited with a public museum collection whenever possible (e.g., Museum of Natural Sciences, Ottawa, Ontario, K1A 0M8). The laboratory museum collection should be maintained in 70% ethanol or isopropanol according to appropriate procedures (Levi, 1966). This collection is invaluable for reference and verification purposes.

   f) comprehensive and updated taxonomic literature should be maintained in the laboratory for reference during the identification process.

   g) inter-laboratory comparison studies may be conducted to establish or verify the level of performance of a laboratory or taxonomist and to provide a comparison of proficiencies of participating laboratories. For example, Ellis and Cross (1981) have described a protocol for inter-laboratory comparisons of biological species identifications or "Ring tests".

Since precise taxonomic identifications are necessary for reliable analyses of communities, it is recommended that a skilled team of taxonomists and collaborating specialists be employed, and appropriate guidelines for quality control observed.

6.2.4 Community Analyses

6.2.4.1 Introduction

Potential environmental consequences of exploration and development activities in coastal areas has prompted "baseline" and "monitoring" studies of potentially sensitive marine areas. Whenever any forms of pollution or environmenal impact are considered, the ultimate or long-term consequences are biological in nature, despite the many temporary changes which might occur in the physical and chemical characteristics of the water column and sea bed.

The basis of the ecological approach is that any significant introduction (or removal) of any substance or energy which interacts with biological processes must cause changes in structure, dynamics and energy flow within the ecosystem (Odum, 1971; Stirn, 1981). Organisms may display limited capacities to assimilate deleterious substances and considerable resiliency to environmental change which may allow re-establishment of equilibria. However, the most critical effects of environmental degradation are the biological disorders which lead to secondary changes in the environment and in biological communities. Such ecosystem modifications are theoretically retraceable in the structures and functions of communities. In the study of coastal areas, this is a compelling rationale for studying the nature of benthic communities and for collecting data that will allow testing of hypotheses regarding temporal (e.g. predisturbance/post disturbance, seasonal) and spatial (e.g. habitat, location) changes to the distribution and structure of benthic communities.

The study of communities of marine benthos began with Petersen (1913) who recognised that relatively uniform combinations of macrofauna species, with a few being dominant in abundance, were found in quantitative grab samples from wide areas of flat sea bottom (other combinations inhabited other level bottom areas in similar uniform ways). The idea of statistical community units developed from the frequent observations on the constancy and uniformity of these large bottom flats and their characteristic species assemblages. The most numerous, conspicuous, and for the area most "characteristic" members of the species group were used to designate the community (Petersen, 1913; Thorson, 1957b).

Petersen's concept of marine benthic communities, together with related concepts from terrestrial plant ecology (Whittaker, 1962; Mills, 1969) have become widely accepted, but their uniformity has been challenged. Petersen's method of classifying the communities also has been criticized as being too subjective. More objective, multivariate statistical methods of classifying species groups have since been developed for analysis of community associations. These methods include:

1) recurrent group analysis (Fager, 1957; 1963),
2) cluster analysis (e.g. Sokal and Sneath, 1963; Williams, 1971; Popham and Ellis, 1971),
3) trellis method (Sanders, 1960; MacFayden, 1963; Stirn, 1981),
4) ordination (e.g. Hill, 1973; Gauch et al., 1977), and
5) correspondence analysis (Hill, 1974; Greenacre and Degos, 1977; Greenacre, 1978).
6) multivariate analysis of variance (ANOVA) and the related methods of canonical analysis and multiple discriminant analysis (Cooley and Lohnes, 1962; 1971; Pimentel, 1978).

This review will describe some features of cluster analysis, the trellis method and ordination. Green (1979) provides a useful review of multivariate techniques, including cluster analysis, ordination, multivariate ANOVA, canonical analysis and multiple discriminant analysis. A guide to use of these techniques for the design and analysis of applied environmental studies is also provided in Section 5, Volume 2. Before a data set is analysed statistically, techniques for screening the data are generally applied for gross violations of assumptions of the methods to be used (e.g. Green, 1979).

Data transformations (e.g., y = log x), which change the scale of measurement so that the variances are independent of the mean values, are a remedy for the situation of heterogeneity of error variances. These topics are discussed in detail in Green (1979) and in Section 5, Volume 2.

6.2.4.2 Cluster Analysis and the Trellis Method

Cluster analysis is a general term which applies to a large class of numerical techniques for classifying related elements (e.g. species, stations) into groups based on high measures of similarity (Sokal and Sneath, 1963). Clustering methods are suitable when the working hypothesis is that "the population of elements can most profitably be regarded as comprising an unknown number of partly-dissociated sub-populations" (Williams and Lance, 1968). The issue of whether biological communities are continuous or discrete has not been resolved (see Mills (1969) for a review regarding marine benthic communities and Whittaker (1962) regarding terrestrial plant communities). There is, however, a general consensus that success in employment of clustering or ordination procedures does not depend on the outcome of this debate (Lambert and Dale, 1964; Goodall, 1973). The principles of clustering or classification are reviewed by Sokal and Sneath (1963) and Williams (1971). The latter review includes an hierarchical examination of clustering strategies. General references on cluster analysis are presented by Anderberg (1973) and Duran and Odell (1974). The user's manual for the CLUSTAN package of computer programs (Wishart 1975) considers strategies for choosing a clustering method appropriate to the situation.

A brief description of common clustering procedures follows; some of the terms used in cluster analysis and ordination are defined in Table III-6.1. The aim of clustering in ecological studies is to probe for intergroup boundaries which possibly indicate environmental discontinuities (Williams, 1971). The similarity of samples by their species composition (Q-type "normal analysis") or of species by their distribution in samples (R-type "inverse analysis") may be determined by a given clustering procedure (e.g., Popham and Ellis, 1971). Most clustering methods first group those elements most related, and gradually admit more members into a cluster by lowering the criteria of admission (i.e. level of similarity coefficient). Admission of an element or a cluster into another cluster can be determined by several linkage criteria, such as single linkage, average linkage and complete linkage (see Sokal and Sneath (1963) for a discussion). The commonly used unweighted pair-group method employs average linkage, which bases admission of an individual into a cluster on the average of the similarities of that individual with the members of the cluster. The unweighted pair-group method permits only one entity to join a cluster during any one computational cycle: the one with the highest average similarity value with the cluster. After all prospective members have joined their clusters, a new similarity matrix of all clusters with each other and with single stems is recalculated.

Another option of clustering methods is to introduce a weighting factor for the order of admission of individuals to a cluster. In the weighted method, each individual carries as much weight as the entire cluster to which it is being added in determining its relation to that cluster. In the unweighted method, all individuals are weighted equally throughout the clustering procedure. Sokal and Rohlf (1969) found that among four clustering methods tested, including weighted and unweighted pair-group methods, the latter gave the least distortion of the original correlation coefficients. Rohlf (1974) discussed aspects of comparing two or more cluster analyses.

TABLE III-6.1

DEFINITIONS USED IN COMMUNITY ANALYSES

Term	Definition	Reference
Cluster	a group of associated elements (species, samples, stations, etc.)	Sokal and Sneath (1963)
Community	a group of organisms occurring in a particular environment, presumably interacting with each other and with the environment, and separable by means of ecological survey from other groups.	Mills (1969)
Dendrogram	a graphical representation of affinities between clustered elements which are indicated as branching stems. (Figure III-6.1)	
Linkage	refers to the criteria by which members are admitted to a cluster. 1) "single" linkage allows admission to a cluster if the element in question is associated with any member of that cluster at a given similarity level. 2) "average" linkage bases admission of any individual into a cluster on the average of the similarities of that individual with the members of the cluster.	Sokal and Sneath (1963)
Similarity coefficient (index)	an interindividual measure of "likeness" or affinity (see Table III-6.2 for examples)	Sokal and Sneath (1963) Williams (1971)

There are many possible similarity indices (interindividual measures) which can be used in cluster analysis (see Williams, 1971; Sokal and Sneath, 1963, for discussion). Three main classes of indices have been described by Williams (1971):

1) measures based on the first Minkowski metric of the basic form

$$|x1j - x2j| ;$$

2) the second Minkowski metric (Euclidean distance)

$(|x1j - x2j|^{2})^{1/2}$ or its square; and

3) various forms of information statistic using the formulation of Shannon (n ln n).

The Jaccard and Czekanowski (Bray-Curtis) coefficients (Table III-6.2) of type (1) have been frequently used in benthos studies (e.g., Popham and Ellis, 1971; Heath et al., 1981; Maurer and Leathem, 1981).

The results of clustering procedures are presented as a dendrogram (Figure III-6.1) or as a similarity matrix (also called a trellis diagram; Figure III-6.2). In a dendrogram, the relationships between elements are indicated by a grouping of stems at various levels of the similarity index. In a trellis diagram, the relative affinities of matched pairs of elements may be shown by values of the similarity index in the "trellis" below the matrix diagonal, and by patterns of shading in the "trellis" above the diagonal (Figure III-6.2).

The trellis method for estimating faunal affinities (Stirn, 1981) is related to cluster analysis in that the trellis diagram or similarity matrix can be used to illustrate results for both techniques, and in both methods, similarity coefficients or indices (e.g., Jaccard's coefficient) are used to express the affinities between compared samples. The trellis method, however, is based on a matrix procedure rather than a sorting algorithm as in cluster analysis.

Sanders (1960) used the trellis method to show the affinities between benthos samples based on the percentage composition of various species in each sample. The samples were arranged at right angles along the ordinate and abcissa, and all possible pairs of samples were compared according to their shared species composition. The resultant value, Sander's index of affinity, is a measure of the percentage of the

TABLE III-6.2

EXAMPLES OF SIMILARITY INDICES

INDEX NAME	EQUATION	SYMBOLS	DATA TYPE	REFERENCES
Jaccard's coefficient of community	$J_C = \frac{c}{a + b - c}$	a = no. of species in sample A b = no. of species in sample B c = no. of species in common	Presence - Absence or Quantitative	Sokal and Sneath, 1963 Popham and Ellis, 1971
Bray-Curtis (Czekanowski) index	$C_Z = \frac{2W}{a + b}$	W = sum of lesser values of the species scores in samples A and B a = sum of species score in sample A b = sum of species score in sample B	Quantitative or Log (x + 1) transformed Quantitative	Bray and Curtis, 1957 Heath et al., 1981 Maurer and Leathem, 1981

Bray-Curtis Co-efficient Unweighted Pair-Group Clustering Log Transformation of Data

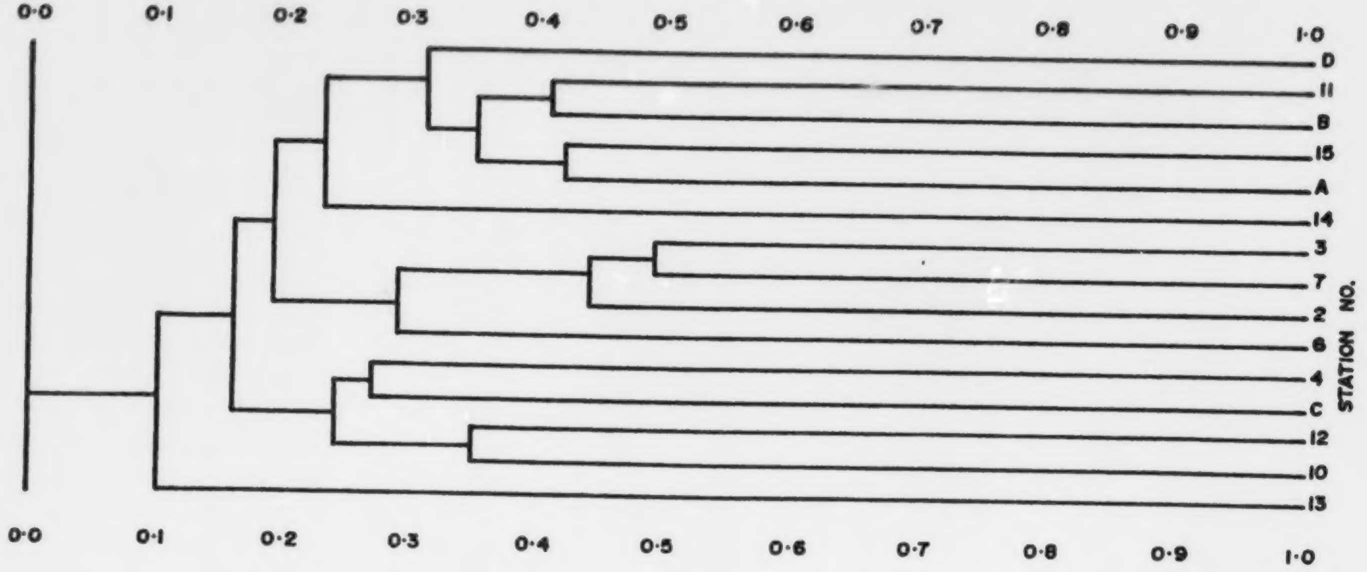
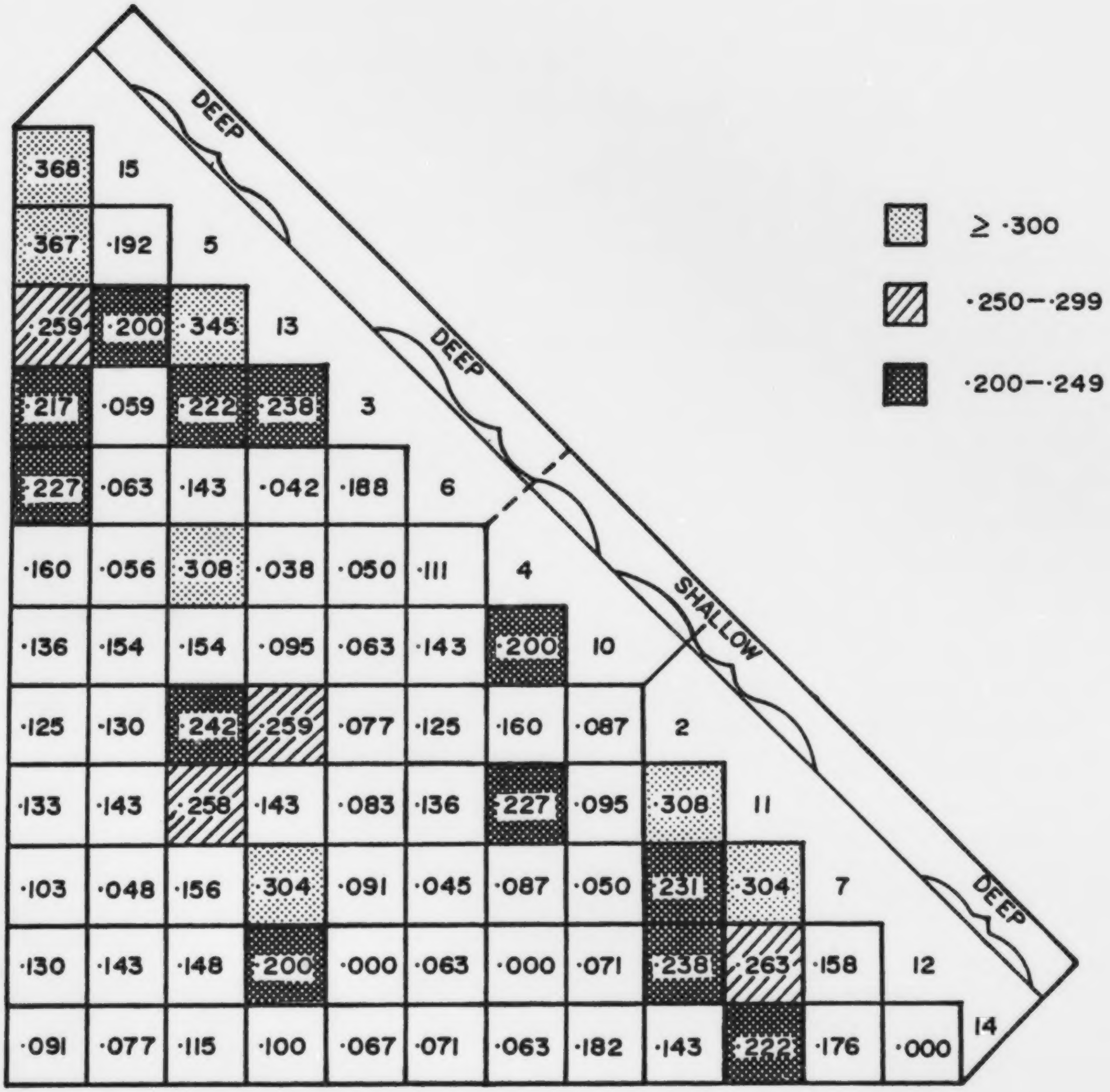


Figure III-6.1 Dendogram of a cluster analysis based on the Czekanowski co-efficient (from Thomas et al., 1981; data from Tuktoyaktuk Harbour).



·368	15											
·367	·192	5										
·259	·200	·345	13									
·217	·059	·222	·238	3								
·227	·063	·143	·042	·188	6							
·160	·056	·308	·038	·050	·111	4						
·136	·154	·154	·095	·063	·143	·200	10					
·125	·130	·242	·259	·077	·125	·160	·087	2				
·133	·143	·258	·143	·083	·136	·227	·095	·308	11			
·103	·048	·156	·304	·091	·045	·087	·050	·231	·304	7		
·130	·143	·148	·200	·000	·063	·000	·071	·238	·263	·158	12	
·091	·077	·115	·100	·067	·071	·063	·182	·143	·222	·176	·000	14

Figure III-6.2 Trellis diagram showing degree of similarity (Jaccard coefficient of community; from Thomas et al., 1981).

fauna common to a pair of samples. It is obtained by totalling the smaller percentage of those species present in both samples. For instance, if species S represents 20% of Sample I and 30% of sample J, then 20% of the total affinity between the samples is contributed by species S. The process of comparison is repeated for all shared species to arrive at the resultant value of the index of affinity. The matrix is then rearranged to bring the samples with the highest values of the index close together, thus grouping the samples that are most closely related in species compositon. Besides indicating which stations have high affinities, the trellis method shows the degree of similarity between replicate samples which reflects sampling variability or possibly faunal heterogeneity. Stirn (1981) has provided examples of applications of the trellis method in ecological assessment of pollution effects. The trellis method has been criticised as being time consuming, subjective and insensitive (e.g., Hughes and Thomas, 1971b), especially when dealing with the large data sets.

The nature of deterministic similarity techniques such as the trellis method and cluster analysis makes objective interpretation of the station or species groups uncertain (Carey, 1980). The major difficulty is the lack of a null hypothesis against which the results may be tested (Connor and Simberloff, 1978). The credibility of the species (or station) groups depends on the differences in the coefficient of similarity having objective significance; however, this objectivity cannot be demonstrated (Connor and Simberloff, 1978). Dissatisfaction with the arbitrary nature of similarity indices and clustering methods has led to a proposal for alternative approaches to studying compositional similarity, such as those based on a probabilistic hypothesis (Harper, 1977; Simberloff, 1978; Raup and Crick, 1979). Details and examples on the probabilistic approach to compositional similarity are presented by Connor and Simberloff (1978) and Carey (1980).

6.2.4.3 Ordination

Rather than attempting to partition species or stations (samples) into distinct groups or assemblages, ordination techniques treat distributions of individuals as continua by plotting the individuals along axes of variation in the data (Goodall, 1954), which often correspond to gradients of environmental parameters (Whittaker, 1967). Ordination methods express the observations in terms of as few variables as possible while still maintaining the integrity of the data (Green, 1979). There are two major approaches to the use of ordinations (Gauch, 1977):

1) Ordinations may be used primarily as a method of reducing the dimensionality of a data matrix so that the structure can be graphed and inspected. A resulting scatter figure expresses the affinities of the ordinated samples or species, and permits observations of trends in related characteristics. Ordinations of this type are "single-space" ordinations in the sense that they represent the positions of the entities in a space directly defined by the variables (e.g., a species-dimensional space in which species scores are axes and samples are points in the space). These ordinations are referred to as indirect or taxometric ordinations (Gauch et al., 1977).

2) Ordination procedures may be used to relate samples and species to envirionmental gradients and to examine patterns of communities in relation to patterns of environmental factors. Ordinations of this type are "dual-space" ordinations in the sense that they represent the positions of the entities in a space defied by variables other than those used for ordination parameters: i.e., environmental gradients rather than species scores or similarity measures. These ordinations, therefore, aim to make inferences about environmental relationships of samples and species, rather than merely summarizing the measurements themselves. Such ordinations are referred to as direct or ecological ordinations (Gauch et al., 1977). For successful application, direct ordination techniques must be accommodated to the curvelinearities of ecological data (i.e., the bell-shaped or Gaussian distribution of species populations along environmental gradients, and the nonlinear relationship of similarity values to environmental separation (Gauch et al., 1977). The basis and associated problems of direct ordination have been discussed by Whittaker (1967), Whittaker and Gauch (1977) and Austin (1977).

The four most commonly used techniques of ordination are principal components analysis (PCA), weighted averages, (WA), polar ordination (PO) and reciprocal averaging (RA). These methods have been compared by Gauch et al., (1977) and a flexile computer program which incorporates all four of the techniques has been described by Gauch (1977). One of these methods, RA (Hill, 1973), is described below.

Reciprocal averaging (RA) may be described as a weighted-average ordination obtained by successive approximations which reveal correspondences between two types of information, such as species and samples (Hill, 1973; Gauch et al., 1977). According to the "direct iteration" procedure as presented by Hill (reproduced in Appendix E as a worked example), species are weighted by positions along a proposed initial gradient and the weights are used to compute sample scores. These sample scores as weights are then used to derive a new and better calibration of the species. In return, the new species weights are used to improve the precision of the sample scores and so on. Consequently, the iterative calculations converge to a stable, optimal solution that does not depend on the initial arrangement. The process is called 'reciprocal averaging' because the species-scores are averages of the sample-scores and reciprocally the sample-scores are averages of the species-scores. It follows that, for reciprocal averaging species ordinations and sample ordinations come in dual pairs, neither of which has logical dominance (Hill, 1973). Gauch et al. (1977) compared the effectiveness of RA, principal components analysis (PCA) and polar ordination (PO) under a wide range of data set conditions. They concluded that RA is a preferred method for indirect ordination (based on species distributions alone) for revealing major direction of sample variation in response to environment. The method is heuristic and its results can be useful in forming hypotheses about the distribution and abundance of organisms in relation to environmental variables.

The relative advantages of RA and PCA have also been discussed by Tuxen (1973).

Examples of the use of ordination in benthic analysis are presented in Cassie and Michael (1968), Lie and Kelley (1970), Hughes and Thomas (1971a and b), and Conlan and Ellis (1979).

Each of the four common ordination techniques (RA, PCA, WA and PO) have different advantages and disadvantages, so that a joint strategy which makes use of all of them can be most effective; the ORDIFLEX program facilitates this approach (Gauch, 1979). In addition, several successive ordination trails may be necessary to clarify environmental relationships in the data set (Hill, 1973).

6.2.4.4 Summary

The methods of community analysis reviewed here all have certain advantages and limitations. The **trellis method** is simple, yet tedious to apply to large data sets. Since many of it's operations require decisions by the user, it tends to be more subjective, and less sensitive than other methods. The trellis diagram can be an effective means of displaying faunistic associations inferred from the trellis method and from cluster analysis. Clustering procedures can be useful for identifying assemblages which may have ecological significance. The approach is primarily useful for description or hypothesis erecting rather than for hypotesis testing. Similarly, indirect ordinations are useful for data reduction and description, and for hypothesis erection concerning faunal-environmental relationships (e.g., Flint, 1981). Direct or ecological ordinations are suitable for relating biotic variables to environmental factors in order to make inferences about environmental relationships of samples and species. The techniques of accommodating the ordination procedures to the curvelinearities of ecological data require care and finesse. The choice of a particular technique of multivariate analysis will depend on the purpose, the hypothesis and the sampling design of a particular benthos study. The analysis used should be appropriate to the information required and to the manner in which the data were collected. An efficient technique of statistical analysis will be as conservative, powerful, and robust as possible (Green, 1979). Being conservative implies that it will have a low probability α of making a Type I error (Section 3.1.3). If it is powerful, it will have a low probability β of making a Type II error. If it is robust, it will not be seriously compromised by the nature of data often encountered in environmental studies. Robustness of environmental decisions reached by statistical analysis will be increased by the application of two or more analysis methods based on different assumptions (Crovello, 1970; Green, 1979). This approach may be particularly appropriate when conducting exploratory analyses on multivariate data sets where hypotheses are only roughly framed (Green, 1979).

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7. SELECTION, COLLECTION AND MAINTENANCE OF ORGANISMS FOR BIOASSAYS

7.1 Introduction

A bioassay generally involves the exposure of organisms in the laboratory to various substances in an attempt to predict whether the disposal of those substances in the environment will cause adverse effects at environmentally realistic levels of the substances. "Bioassay" has been defined in several ways:

(1) "a test in which the quality or strength of a material is determined by the reaction of a living organism to it" (Sprague, 1973);

(2) "a procedure in which the responses of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combination" (APHA, 1980);

(3) a test estimating "the amount of biologically active substances by the level of their effects on organisms" (Chapman and Long, 1983).

A key factor in any successful bioassay is the collection and subsequent maintenance of healthy organisms. This section reviews the types of arctic species that have been and can be used for bioassays and reviews methods that have been used to both collect and maintain these organisms. A general review of bioassays, terminology, uses, limitations and data presentation is given as Appendix F.

7.2 Selection of Species for Bioassays with Special Reference to Arctic Conditions

It is apparent from the literature that no single species is suitable for all bioassay tests. Further, it would appear that many workers are recommending that a variety of organisms either representative of various trophic levels, (Maciorowski and Clarke 1980) or of various niches or phlya (Engler, 1980) be used in assay studies. The selection of the test species can be made after consideration of the following criteria (Anon, 1978; Shuba et al., 1981; Buikema et al., 1982) ordered on the basis of importance regarding the use of arctic species.

1) they are either ecologically important or economically important representatives of the habitat where the potential toxicant is likely to reside;

2) the species are representative of various trophic levels;

3) they can be reared in the laboratory or easily maintained;

4) the species are available through collection or purchase;

5) they are sensitive to a wide variety of substances;

6) a toxicological data base exists for the species;

These criteria are often mutually exclusive and can be weighted according to the objectives of the study. A selection of different objectives would lead to a selection of different species. An economically important species could be used for comparing the relative toxicities of various substances while an easily maintained species would be appropriate to measure the effects of a single toxicant over time. A sensitive species could be used as a screen to determine whether a new substance would have serious environmental impacts. A flow chart for selecting test organisms is shown in Figure III-7.1. A summary of species previously used in the Arctic for bioassays or bioaccumulation studies is given in Table III-7.1.

However, it should be kept continuously in mind while reading the next section that a strong case can be made for the notion that arctic species are not necessarily more sensitive to exposures to toxicants than are cool temperate species (Percy and Wells, 1984).

7.2.1 Bioassays Using Algae

Algae form the base of most marine food webs. Changes or reduction in algal populations, particularly macroalgae, as a result of marine pollution could lead to a change in the community of organisms at the site of impact. Hence, algal bioassays are useful in the following applications for predicting possible effects of discharges of pollutants into a marine ecosystem: 1) routine screening of potential toxicants, 2) evaluation of water quality; and 3) monitoring the biological impact of suspended solids.

It would appear that algae, especially freshwater species, have been used frequently (e.g., Wong et al., 1983; Van Coillie et al., 1983) for assessing

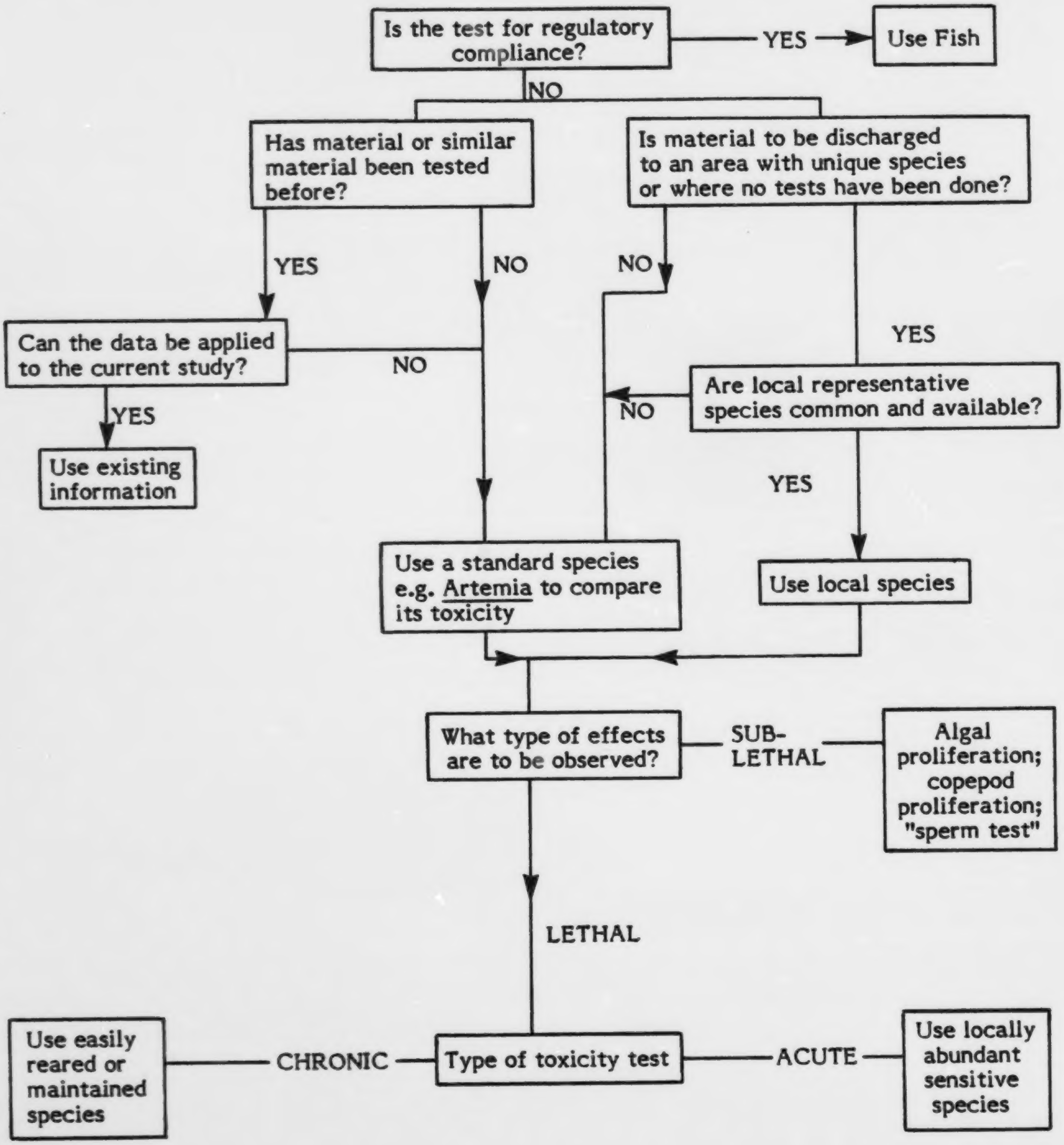
Is the test for regulatory compliance?
YES
Use Fish
NO
Has material or similar material been tested before?
Is material to be discharged to an area with unique species or where no tests have been done?
YES
NO
NO
YES
Can the data be applied to the current study?
NO
YES
Use existing information
Are local representative species common and available?
NO
YES
Use a standard species e.g. Artemia to compare its toxicity
Use local species
What type of effects are to be observed?
SUB-LETHAL
Algal proliferation; copepod proliferation; "sperm test"
LETHAL
Use easily reared or maintained species
CHRONIC
Type of toxicity test
ACUTE
Use locally abundant sensitive species

Figure III-7.1. Flowchart for selecting test organisms for bioassays.

Table III-7.1

Arctic Species Used in Biological Assays and Bioaccumulation Studies

Taxa	Species	Type of Test	Reference
Coelenterata	Haliothlus cirratus	oil/acute toxicity	Percy and Mullin, 1975
Crustacea			
Amphipoda	Onisimus (=Boeckosimus) affinis	oil/acute toxicity	Percy and Mullin, 1975
	Corophium clarencense	oil/acute toxicity	Percy and Mullin, 1975
	Atylus carinatus	oil/acute toxicity	Percy and Mullin, 1975
	Gammarus oceanicus	oil/sub lethal toxicity	Percy, 1976
	Gammarus zaddachi	oil/acute toxicity	Bushdosh and Atlas, 1977
	Anonyx nugax	oil/acute toxicity	Schnieder, 1980
	Orchomene pinguis	oil/acute toxicity	Rice et al., 1979
	Haploops laevis	trace metal bioaccumulation	Thomas, 1977, 1978a
	Amphipoda	in-situ metal bioaccumulation	NORTEC, 1981
	Amphipoda - several species (Weyprechtia pinguis Onisimus litoralis O. glacialis Gammarus setosus Ischyrocerus sp. etc.)	oil and dispersed oil/in situ effects on under-ice amphipod community	Cross, 1982; Cross and Martin, 1983
Cirripeda	Balanus crenatus	oil/acute toxicity	Percy and Mullin, 1975
Cumacea	Brachydiastylis resima	oil/acute toxicity	Percy and Mullin, 1975
Decapoda	Hemigrapsus nudus	oil/sub lethal response	Rice et al., 1977
	Paralithodes camtschatica	oil/chronic toxicity	Mecklenberg, et al., 1977
Isopoda	Saduria entomon (= Mesidotea entomon)	oil/acute toxicity	NORTEC, 1981
	Mesidotea entomon	oil/acute toxicity	Percy, 1976, 1978
	Mesidotea entomon	trace metal accumulation	Thomas, 1978a, b
	Mesidotea sibirica	oil/sub lethal toxicity	Percy, 1977
Natantia	Eualus suckleyi	oil/chronic toxicity	Rice et al., 1977
	Pandalus hypsinatus	oil/chronic toxicity	Mecklenberg, et al., 1977
	Pandalus platyceros	oil/sub lethal response	Malins, et al., 1977
Mysida	Mysis relicta	oil/acute toxicity	NORTEC 1981
	Mysis littoralis	oil/acute toxicity	Schnieder, 1980
	Acanthamysis pseudomacropsis	oil/acute oxicity	Rice et al., 1979
Mollusca			
Bivalvia	Macoma balthica	oil/acute toxicity	Rice et al., 1977
	Mytilus edulis	oil/acute toxicity	Rice et al., 1979
	Protothaca staminea	oil/acute toxicity	Rice et al., 1979
	Macoma calcarea	trace metal bioaccumulation	Thomas, 1977, 1979
	Astarte borealis	trace metal bioaccumulation	Thomas, 1977
	Mya truncata	metal bioaccumulation	Fallis, 1982, Thomas et al. 1983
	Mya truncata	dispersed crude oil/ behavioural response	Engelhardt et al., 1983, Mageau and Engelhardt, 1984
	Serripes groenlandicus	dispersed crude oil/ behavioural response	Engelhardt et al., 1983; Mageau and Engelhardt, 1984
Gastropoda	Natica clausa	oil/acute toxicity	NORTEC, 1981
	Neptunea sp.	oil/acute toxicity	NORTEC, 1981
	Buccinum sp.	oil/acute toxicity	NORTEC, 1981
	Margarites populis	oil/acute toxicity	Rice et al., 1979
	Littorina sitkana	oil/acute toxicity	Rice et al., 1979
	Thais lina	oil/acute toxicity	Rice et al., 1979
	Colus balli	oil/acute toxicity	Rice et al., 1979
	Neptunea lyrata	oil/acute toxicity	Rice et al., 1979
Polychaeta	Melaenis loveni	oil/acute toxicity	NORTEC, 1981
	Nereis vexillosa	oil/acute toxicity	Rice et al., 1979
	Pectinaria sp.	trace metal bioaccumulation	Thomas, 1977
Echinodermata	Strongylocentrotus droebachiensis	trace metal bioaccumulation	Bohn, 1979, Fallis, 1982
	Ctenodiscus crispatus	trace metal bioaccumulation	Thomas, 1977
	Strongylocentrotus droebachiensis	dispersed crude oil/ behavioural response	Engelhardt et al., 1983; Mageau and Engelhardt, 1984
Vertebrata			
Pisces	Oncorhyncus kisutch	oil/sublethal effect	Malins et al., 1977
	Oncorhyncus tshawytscha		
	Myoxocephalus quadricornis	oil/acute toxicity	Percy and Mallin, 1975
	Coregonus nasus	oil/acute toxicity	NORTEC, 1981
	Boreogadus saida	oil/acute toxicity	NORTEC, 1981
	Eleginus navaga	oil/acute toxicity	NORTEC, 1981
	Coregonus autumnalis	oil/acute toxicity	NORTEC, 1981
	Myoxocephalus verrucosus	oil/acute toxicity	Devries, 1977
	Megalocottus platycephalus laticeps	oil/acute toxicity	Devries, 1977
	Myoxocephalus scorpius	metal bioaccumulation	Bohn and Fallis, 1978
	Salvelinus alpinus	metal bioaccumulation	
	Salvelinus alpinus	mercury bioaccumulation	Smith and Armstrong, 1975
Mammalia	Phoca phoca hispida	mercury bioaccumulation	Smith and Armstrong, 1975
	Erignathus barbatus	mercury bioaccumulation	Smith and Armstrong, 1975
	Thalarctos maritimus	mercury bioaccumulation	Eaton and Farant, 1982
	Thalarctos maritimus	polychlorinated biphenyls	Bowers and Jonkel, 1975
Aves	Larus glaucescens	oil/toxicity	Patten and Patten, 1977
Algae	Fucus distiches	metal bioaccumulation	Bohn, 1979
	Fucus vesiculosus	metal bioaccumulation	Fallis, 1982
Microalgae	Nitzschia grunowii	oil and dispersed oil/in situ effect on primary productivity	Cross, 1982
	N. frigida		Cross and Martin, 1983

environmental toxicological effects. Murphy et al. (1983) used single cell isolates of species of a number of different representative families of algae to investigate the effects of industrial wastes on their growth. They were able to run 100-300 tests simultaneously because of the compact nature of the test. Methods for culturing algae can be found in the text edited by Stein (1973).

Standard Methods (APHA, 1980) describe a tentative method for the use of algae and recommends that Dunaliella tertiolecta, Thalassiosira pseudonana (sensitive to heavy metals), and Skeletonema be used for toxicity tests for marine environments. These species are readily obtained as standard laboratory cultures, but may be more appropriate to use locally available species. Gentile and Johnson (1974) list some guidelines for selecting an appropriate species:

1) Select indigenous species representing a diversity of phylogenetic types from the major seasonal successions.
2) Select the more sensitive species among the range of species.
3) Identify the conditions of greatest vulnerability.
4) Test species and culture conditions should yield 0.5 to 1 doubling per day.

One possible way to ensure comparability is to use standard reference toxicants to indicate the relative sensitivity of the field-collected sample vis-a-vis laboratory-reared specimens.

Wong et al. (1983) in a comparison of a number of techniques have shown that;

1) static (batch) bioassays for heavy metal mixtures using uptake of C14-labelled sodium bicarbonate is a sensitive, short-term (four hours) test which reduces the problem of bacterial contamination of the culture;

2) that measurements of cell growth (long term; 30 days) using optical density is convenient and allows sufficient time for the cells to become adapted to the conditions; and

3) that direct counting of cells is extremely simple and costs little to undertake and allows the investigator to observe any morphological changes that have taken place.

However, they point out a number of disadvantages to these three techniques, including cost, absence of a long-term response, insensitivity, the problem of autoinhibition and the tedium of counting. They found that continuous culture techniques using either a turbidostat and a chemostat allowed the experiment to be run under more natural conditions. However, a long time was required to set up the systems and there were difficulties in preventing algal growth along the walls of the test chamber.

Van Coillie et al. (1983) have pointed out that algae are also useful organisms for studying sublethal toxic effects including such phenomena as mutagenesis (normally applied on bacteria by such procedures as the Ames test), bioaccumulation and biotransformation of toxic compounds. Stockner and Antia (1976) have shown that short-term bioassays using algae may be unreliable because of a "shock" response when introduced to a new medium. They recommend that long-term (30-40 days) bioassays be undertaken with phytoplankton in order to allow them to acclimate to the new conditions.

7.2.2 Bioassays Using Ciliated Protozoa

The advantages to using ciliated protozoa for toxicity tests are: 1) they are easily maintained and require little capital expenditure, 2) they have rapid generation times, 3) they are the most numerous organisms in the benthos, and 4) they contribute to the recycling of nutrients (Sleigh, 1973). One major disadvantage, however, is that a high degree of expertise and a specialised laboratory are required.

Standard Methods (APHA, 1980) describes a procedure for the ciliated protozoan, Tetrahymena pyriformis, as this species has been extensively studied, can be kept in a bacteria-free (axenic) culture, and has a world-wide distribution. Recently, Slabbert and Morgan (1982) have shown that by measuring over a ten minute period the respiratory response of T. pyriformis exposed to toxicants, it is possible to develop a sufficiently sensitive technique to monitor the toxicity of industrial effluents. Persoone and Dive (1978) review a variety of methods used for toxicity testing with ciliates. Local varieties of ciliates can be isolated by following the procedures described in Gray (1974) and Lee and Muller (1974). Dive and Leclerc (1975) have published a more detailed account of conducting tests than given in Standard Methods (APHA, 1980).

7.2.3 Bioassays Using Polychaetes

Polychaetes form an important component of arctic marine benthic ecosystems and may be an important food source for fish and large crustaceans. Standard Methods (APHA, 1980) lists a number of temperate species that are recommended as test organisms although almost all of them appear not to be found in the Arctic. However, a great variety of species are found in the Arctic and one of them, Melaenis loveni, has been used as an assay organism to test the toxicity of drilling fluids. If local specimens are not available then the species Ophryotrocha labronica could be considered, as it has been shown to be useful for detecting mutagenic compounds (Bogaert et al., 1983). The trochophore larva of Capitella capitata has been successfully used as a bioassay organism for testing the toxicity of whole sediments and elutriates of sediments (Chapman and Fink, 1984).

7.2.4 Bioassays Using Nematodes

Nematodes have not been listed in Standard Methods but nevertheless they may also prove to be viable assay organisms for determining sublethal effects. The advantage of these organisms is that some are easy to culture, have rapid generation times and are sensitive to chemicals. Samoiloff et al. (1980) describe a technique using the nematode Panagrellus redivivus as an assay organism for mutagens and Tietjen and Lee (1984) describe a method for monitoring growth rates of two species of nematodes exposed to pollutant-contaminated sediments.

7.2.5 Bioassays Using Marine Crustacea

Both Standard Methods (APHA, 1980) and Gentile et al. (1974) describe in detail procedures for using the calanoid copepod, Acartia tonsa. The female of this species is capable of producing in excess of thirty eggs per day and the generation time is seventeen days. This species can be mass cultured in the laboratory using a static system.

Copepods that can be found in the Arctic include: Calanus, Metridia, and Pseudocalanus. Pseudocalanus minutus which is common, has a circumpolar distribution and is easy to maintain (P. Wells, personal communication).

The amphipod *Amphithoe valida* and the isopod *Sphaeroma quadridentatum* are other crustacea which have been successfully reared in the laboratory (Lee 1977) with life cycles of 5-6 weeks and 3.5-5 months respectively. Lee *et al*. (1977) found that *A. valida* and another amphipod which could be raised in the lab, *Gammarus mucronatus*, were more sensitive to aqueous extracts of two petroleum oils than were the benthic polychaetes and shrimp for which there was published data. Swartz *et al*. (1982) used the amphipod *Rhepoxynius abronius* (*Paraphoxysis epistomas*) which burrows into sediments as an assay organism to test the toxicity of sediments. In the Arctic the following three amphipod species have been used as test organisms: *Onisimus littoralis*, *O. glacialis* and *Boeckosimus affinis* (Percy and Mullin, 1975; NORTEC, 1981). In addition, the isopod, *Saduria entomon*, and the mysid, *Mysis relicta*, also have been used successfully (NORTEC, 1981).

The shrimp, *Crangon septemspinosa*, was used by McCleese and Metcalfe (1980) to investigate the toxicities of organochlorine compounds in marine sediments.

The brine shrimp *Artemia salina* is not an endemic marine crustacean but nevertheless it is a useful test organism for assessing the effects of toxicants considering that it is easily obtainable and raised, that there is an extensive data base on its biology, and that it has been used in inter-laboratory comparisons of methods (Vanhaecke and Persoone, 1981). The species has been used to test the comparative toxicity of many materials, such as heavy metals (Saliba and Krzyz, 1976), organic compounds (Kuwabara *et al*., 1980), and oil dispersants (Wells *et al*., 1982; Wells , 1984).

7.2.6 Bioassays Using Molluscs

Molluscs are suitable for both long and short-term tests. A variety of temperate species of oysters, mussels and clams have been suggested by Standard Methods (APHA, AWWA, WPCF 1980), but time to death is difficult to measure accurately in many of the adults. The blue mussel, *Mytilus edulis*, is found in the Arctic and hence may be the most suitable representative at present because the data can be related to previous research studies using the temperate-dwelling forms. A suitable method of using molluscs in short-term tests is to examine either the effect of toxicants on fertilization of oocytes by spermatozoa or on the rate of survival by the embryos and larvae (Cardwell, 1980) instead of determining death in adults. The oyster larvae bioassay technique is also a suitable procedure for evaluating the toxicity of sediments (Chapman and Morgan, 1983). A test procedure for measuring shell deposition has been developed for oysters and this procedure may also have some potential with mussels. A static bioassay of drilling fluids has been performed with the bivalve, Astarte borealis, as the test organism (NORTEC, 1981).

7.2.7 Bioassays Using Fish

Fish are the most frequently used organisms for bioassay procedures and are the required organism for conducting bioassays as a method of regulatory compliance (EPS, 1980). A variety of species found in the Arctic such as the fourhorn sculpin, Myoxocephalus quadricornis, the broad whitefish, Coregonus nasus, and the arctic cod, Eleginus navaga, have been used (NORTEC, 1981). The threespine stickleback, Gasterosteous aculeatus, has been recommended as the test organism to be used in the Arctic (Wong, 1982). In addition, this fish has been used to determine the acute lethality of oil-based drilling fluids to marine fish (EPS, 1985).

7.3 Collection of Organisms for Use in Bioassays

7.3.1 Overview of Sampling Methods for Benthos and Plankton

Healthy organisms are a basic prerequisite for successful laboratory maintenance and subsequent bioassays. Since most collection equipment involves rough handling and often causes injury to the organisms being collected, special attention must be devoted during collection and any consequent handling to reducing the trauma as much as possible and obtaining healthy intact specimens. Sampling for benthos and plankton is generally by indirect or remote means, i.e., by towing (dredges and trawls) or lowering (grabs) collecting gear from a ship. However, organisms are often sparsely distributed and the more mobile members are difficult to sample. Problems may also arise during processing when the researcher attempts to efficiently separate the smaller organisms intact from the sediment. A detailed discussion of methods for sampling the benthic environment is given in Sections II-3 and II-4 as well as in Holme (1964). Refer to Table III-7.2 for a summary of techniques used to collect organisms for bioassays.

Table III-7.2

Summary of Techniques Used to Collect Organisms for Bioassays

Type of Organism Being Collected	Recommended Sampling Technique
Microalgae	
Benthic	In sediment samples obtained with a corer
Pelagic	Plankton nets
Protozoans and other interstitial fauna	In sediment samples obtained with corers and grabs
Nematodes	Grab sampler or divers with suction devices
Polychaetes	Grab sampler or divers with suction devices
Echinoderms	Grab sampler or divers
Crustaceans	Baited traps, divers, beach seines, plankton nets (copepods and mysids). Use grabs, trawls or divers for burrowing species.
Bivalve Molluscs	Grab sampler or divers with suction devices
Fish	Various types of nets, beach seines or divers

Exploratory qualitative sampling of the epifauna has traditionally been conducted with a variety of biological dredges (e.g., clam or oyster dredge) and trawls (e.g., beam, Agassiz and otter). Section II-3.2.1 of this manual provides a detailed explanation of the different types of gear as well as techniques and methods for their use. Most dredges are designed to skim the surface of the bottom. Their main advantage is the relative ease of use even in rough weather. However, sampling by dredge is generally unsatisfactory because only a small fraction of the fauna are sampled and because dredges, unless modified (e.g., bucket or anchor dredge) do not dig deeply enough to sample most of the burrowing infauna.

Trawls are relatively large nets designed to be towed over the bottom. By skimming over the seabed with a large mouth area these devices are effective for collecting larger or scarcer epifauna and fast-moving animals such as bottom living fish, cephalopods and crustaceans. Large commercial otter trawls are not particularly effective for catching smaller animals associated with the bottom fauna. However, smaller versions with liners of finer mesh sizes are widely used from small research vessels in ecological studies of fish and epifauna. Beam trawls are good for collecting inshore stocks of fish, shrimp and prawns whereas the Agassiz, because of its design, does not catch many fish.

The main advantages of dredging and trawling are:

1) the larger, more sparsely distributed or fast-moving epifauna are more likely to be collected, and

2) surface areas of compact and hard substrates can be sampled.

Dredging and trawling in arctic waters is limited to open water conditions.

A grab sampler (e.g., Petersen, van Veen and Smith-McIntyre) is the conventional and best means for quantitatively sampling benthos on soft bottoms. A description of the gear, advantages and disadvantages of various grabs and factors to consider in grab selection are given in Section II-3.3.1. Factors associated with arctic sampling tend to favour the van Veen grab for general use: it is less bulky for shipment, relatively inexpensive, simple to use and can be modified for sampling through small ice holes by shortening the length of the lever arms.

Diving excels as a technique for investigating macrobenthos of hard or gravelly substrates which cannot be adequately sampled by dredge or other means (Section II-4). Hand collection by divers can be a very effective means of obtaining specimens in good condition for laboratory studies. Suction devices combining an airlift and a pump can be used for the careful collection of non-attached forms. Divers may also sample the infauna in soft bottoms with suction devices or assist sampling by various types of corers, box samplers or light grabs.

Sampling the phytobenthos, in particular, benthic marine microalgae, can be accomplished by collecting sediment samples with a corer and then separating the different types (see Section II-5.2.2). Protozoans and other interstitial fauna are collected in sediment samples taken by corers or grabs.

Sediment collected with grabs or dredges must be processed to remove the organisms. Extraction of the infauna usually involves emptying the contents of the dredge or grab onto a sieving table whereby a gentle stream of water from a sprinkler system or hose removes the surrounding sediment, leaving the specimens intact. The water should be the same temperature as the water from which the sample was collected to avoid thermal shock. Gently washing the sample through a nest of screens of various mesh size will aid the recovery of smaller organisms.

Worms (polychaetes and nematodes) are common to most marine benthic environments. They are collected in dredge or grab samples. The worms can be removed from the sediment in the field or can be carried to the laboratory in sediment which acts as a temperature buffer. Either way temperature extremes should be avoided by transporting them to the laboratory in insulated coolers.

Crustaceans (mysids, amphipods, isopods, shrimp and crabs) can be caught from ships, collected in the intertidal zone or obtained through the ice using a variety of gear, including: hand nets, baited traps, or barbless hook and line (using a variety of bait). Beach seines are very effective yet gentle devices for capturing crustaceans in nearshore waters. In addition, crustaceans can be readily obtained in healthy condition by divers usually assisted by suction devices. They can be transported to the laboratory in plastic bags, filled half and half with water and air or in baskets between layers of moist plants or other suitable material.

Many of the adult echinoderms are collected by dredging, especially deep water forms and those which bury themselves. In shallower waters, asteroids and echinoids can be collected by grab samplers or by diving. During transportation echinoderm specimens should be kept cold and damp.

Adult bivalve molluscs are collected by dredge, grab or diver. The adults of many species experience no adverse effects when transported to the laboratory in containers with cold moist toweling. Bivalve larvae for use in bioassays can be obtained by the tedious and often unreliable method of screening living larvae from plankton samples or preferably by collecting adults and obtaining gametes from them for larval culture.

Collection of pelagic organisms like phytoplankton (e.g., diatoms and microalgae), zooplankton (e.g., copepods) and fish is best accomplished by the use of various types of nets (Holme, 1964). Plankon nets equipped with runners can be used to sample the plankton just above the bottom. Beach seines are an effective and gentle means of collecting smaller fish in nearshore waters. Divers can also assist in the collection of fish. It should be noted here that bioassays have shown that the larval forms of many species are more sensitive to environmental contaminants than are the adults. The larvae of many groups (bivalve molluscs, echinoderms, polychaetes, fish) can be obtained by carefully separating them from plankton samples. Since this is a laborious process requiring persons highly trained and experienced in larvae identification the preferred method of procuring early developmental stages is by inducing the adults to spawn naturally in the laboratory and rearing the larvae.

7.3.2 Arctic Studies

Some studies in which arctic species were collected for use in toxicity tests are presented below. These studies illustrate how a variety of techniques and gear are necessary to obtain the required organisms. They also show the variety of arctic organisms which have been used for bioassay procedures as well as indicating to some extent where they may be collected.

Percy has conducted numerous studies, some physiological (Percy, 1975; Percy and Walbridge, 1978; Percy *et al.*, 1978) in nature, others involving bioassay techniques (Percy, 1976; Percy, 1977; Percy, 1978; Percy and Mullin, 1975), utilizing local marine organisms from the western Beaufort Sea. To study the effects of crude oils on arctic marine invertebrates (Percy and Mullin, 1975) eleven species were collected. Seven species (the benthic amphipods, *Onisimus affinis*, *Corophium clarencense* and *Atylus carinatus*; the cumacean, *Brachydiastylus resima*; the barnacle, *Balanus crenatus*; the sculpin, *Myoxocephalus quadricornis*; and the jellyfish, *Halitholus cirratus*) were collected in the vicinity of the Arctic Biological

Station's field laboratory near the entrance to Eskimo Lakes, N.W.T., O. affinis were collected in minnow traps lined with fine nylon screen and baited with fish. C. clarencense, A. carinatus, B. resima and B. crenatus were screened from mud samples obtained with a Petersen grab or a lightweight dredge. The delicate cumaceans were unavoidably damaged during collection and sorting of the bottom samples and suffered a high mortality rate. The medusa, H. cirratus, is also rather delicate and specimens collected with a plankton net were invariably in poor condition. Therefore, all the medusae used in the study were collected in shallow water by scooping them up individually in beakers. Young sculpins were collected in a similar manner. Benthic isopods were collected by otter trawling; Mesidotea sibirica, M. sabini and M. entomon, east of Herschel Island and M. entomon only in 10 metres of water in the vicinity of Tuktoyaktuk. The herbivorous copepod, Calanus hyperborea, was collected in limited quantities by means of vertical plankton tows through leads in the Beaufort Sea pack ice. Percy (1976) has also collected another benthic amphipod, Gammarus oceanicus, with baited modified minnow traps in the brackish water of Eskimo Lakes adjacent to the Mackenzie Delta.

Percy has concentrated the majority of his work on isopods of the genus Mesidotea (M. entomon, M. sibirica and M. sabini) and the amphipod, Onisimus (= Boeckosimus) affinis. O. affinis is a discontinuous circumpolar littoral species whose range extends into subarctic regions. It has been reported from a number of widely separated areas of the Arctic Ocean. In the North American Arctic it has been collected from Demarcation and Collinson Points in Alaska, Ungava Bay and in Eskimo Lakes, adjacent to the Mackenzie Delta in the Northwest Territories. In most instances O. affinis is found in brackish water, coastal areas in the vicinity of river mouths at depths of 0 - 40 metres. It is primarily a benthic detritivore which alternates periods of actively swimming above, and crawling over, the substratum with time spent burrowing shallowly in bottom sediments. Catching the animals in minnow traps lined with fine nylon screen and baited with fish yields adequate numbers of animals in a completely undamaged condition and also proved to be the only feasible method of collection through heavy winter ice (Percy, 1975). The amphipods were transported to the laboratory in insulated containers.

Three isopods of the genus Mesidotea (M. entomon, M. sibirica and M. sabina) are abundant in the southern Beaufort Sea where they are an important component of the benthic community, acting as voracious scavengers and serving as food for a variety of fish and marine mammals. M. entomon pentrates the farthest into shallow coastal waters that are most strongly influenced by the warm, fresh outflow of the Mackenzie river. This species has, in fact, become established in at least one of the fresh water lakes of the delta. The other two species are restricted to the Arctic Ocean. *M. sibirica* occurs in large numbers in deeper marine waters. As well as being collected by trawling *M. entomon* and *M. sibirica* have been collected during July and August in baited traps in Ptarmigan Cove, Herschel Island (Percy *et al.*, 1978). The animals were held one or two days in large polyethylene tanks of aerated seawater at 2° ±2°C before being transported by air in two gallon jugs of cold seawater (2°± 2°C). *M. sibirica* were found to be extremely aggressive when crowded, and had to be shipped individually in sea water in small polyethylene bags. *M. sabini* also occurred in Ptarmigan Cove, however as it was a burrowing form it was not readily caught in baited traps. It was collected during August by bottom trawling in about seven metres of water near the mouth of Liverpool Bay. Freshwater *M. entomon* were collected in July and August in Airport Lake located approximately eight miles south of Inuvik in ten metres of water with baited traps. High mortality occurred in these freshwater amphipods if they were shipped in lakewater or dechlorinated tapwater. However, only a few died if a small quantity (2 °/oo) of Instant Ocean sea salt was added to the medium.

As part of a study to evaluate the effects of drilling effluent discharges from offshore wells in shallow arctic waters, Northern Technical Services (NORTEC, 1981) conducted static 96-hour bioassays upon local organisms from the Prudhoe Bay area of the Beaufort Sea to determine the acute toxicity of whole active drilling mud and cuttings. Sixteen species were selected for use as test organisms either because they were ecologically or economically important species for the Beaufort Sea and/or they were readily available. The suitability of a species for testing depended on the abundance of the species, the ease of capture, the size of organisms and their susceptibility to mortality due to handling and laboratory stress. Some organisms (the Arctic flounder, *Liopsetta glacialis*; the round whitefish, *Prosopium cylindraceum*; the ninespine stickleback, *Pungitius pungitius*; clams and starfish) were never caught in sufficient numbers. Other organisms, including Arctic char (*Salvelinus alpinus*) and least cisco (*Coregonus sardinella*) were frequently caught but were too large to test in 50 liter aquaria. Several other species (a pelagic amphipod, *Gammarus locusta*; boreal smelt, *Osmerus eperlanus*; and Arctic grayling, *Thymallus arcticus*) were unable to withstand handling stress and were impossible to maintain in the laboratory. Winter bioassay test organisms included amphipods (*Onisimus* sp. and *Boeckosimus* sp.), isopods (*Saduria entomon*), polychaetes (*Melaenis loveni*) and gastropods (*Natica clausa*, *Neptunea* sp. and *Buccinum* sp.).

Amphipods were collected in baited traps which consisted of 18 litre plastic buckets with three screened hatches. The hatches were made from vexar cones which were sewn into each opening and tapered inside the bucket to a 3 to 5 cm diameter opening. Bait used in the traps included salmon and shrimp. The traps were lowered through holes drilled in the ice and were positioned on or near the ocean floor. Isopods were obtained in small numbers from the amphipod traps but because the amphipod densities were generally very high the isopods were frequently eaten. The isopods, polychaetes, molluscs and a few fish were collected by divers below the ice. These animals were caught by hand, with the exception of a few Arctic cod caught in a fyke net deployed by the divers.

Fish and mysids were not available in sufficient numbers for testing during the late winter but many species were captured during the summer sampling. Species used as test organisms in the summer bioassays included the fourhorn sculpin (Myoxocephalus quadricornis), the broad whitefish (Coregonus nasus), Arctic cod (Boreogadus saida, Arctic cisco (Coregonus autumnalis), saffron cod (Eleginus navaga) and mysids (Mysis sp.).

Mysids were collected with plankton nets in shallow waters and transported to the laboratory in 18 liter buckets. Fish were captured in fyke nets by positioning a 61 metre lead net with two 15 metre wings perpendicular to the shore. Large, stressed or overly abundant fish were returned to Prudhoe Bay.

In a study (Schneider and Hanes, 1981) to determine the responses of arctic benthic and epibenthic invertebrates to salinity and crude oil stress under winter conditions several local species were collected from two sites near Barrow, Alaska. Benthic infaunal species (the amphipods, Aceroides latipes and Pontoporeia femorata; the isopod, Saduria sabini; the polychaetes, Pectinaria hyperborea and Brada villosa; the bivalve mollusc, Liocyma fluctuosa and a unidentified anemone) were collected with an airlift operated by a SCUBA diver. Using this method of collection usually yielded animals which were not visibily injured and which appeared to be in good physiological condition. A heated dive hut was maintained at each site and upon collection the organisms were transferred immediately to buckets of freshly collected seawater to prevent thermal shock. The epibenthic species (the amphipods, Anonyx nugax and Boeckosimus affinis, and the mysid, Mysis littoralis) were collected with amphipod traps baited with sardines. The traps were usually deployed through dive holes at the sites.

Finally, as part of the Baffin Island Oil Spill (BIOS) Project a variety of species have been utilized to assess the effects of oil and dispersed oil on organisms inhabiting shallow arctic waters. *In situ* studies (Cross, 1982; Cross and Martin, 1983) have been carried out on under-ice amphipod communities (amphipods used in these studies include the following species: *Weyprechtia pinguis*, *Onisimus litoralis*, *O. glacialis*, *Gammarus setosus* and *Ischyrocerus* sp.) and on the primary productivity of the ice algae community (dominated by pennate diatoms, in particular, *Nitzschia grunowii* and *N. frigida*). The sea urchin, *Strongylocentrotus droebachiensis*, and the bivalves, *Serripes groenlandicus* and *Mya truncata*, were collected by divers in 7 metre water depths off the western shore of Ragged Island, a site near Cape Hatt, N.W.T. (Engelhardt *et al*., 1983; Mageau and Engelhardt, 1984) for use as test organisms.

7.4 Laboratory Maintenance of Organisms to be Used in Bioassays

7.4.1 Overview of Maintenance Methods

The successful laboratory maintenance of organisms depends to a large degree upon how well natural environmental conditions can be simulated. There are four fundamentals to successful maintenance: (1) the water (medium) used; (2) the management of its life-supporting qualities; (3) adequate nutrition of the organisms and (4) proper equipment. Special attention must be given to water quality because the life-supporting capacity of seawater removed from the ocean tends to decrease. Major alterations include an increase in ammonia, organic compounds and carbon dioxide, and a decrease in alkalinity, dissolved oxygen and pH. These changes must be counteracted by mechanical, biological or physico-chemical water treatment. Kinne (1976) gives a detailed description of water quality management and technology in relation to the cultivation of marine organisms. Ammonia is of particular importance among the life-endangering substances which accumulate in the water. Biological water treatment is the most effective counter-measure. In addition to microbial and algal water treatment, disinfection of sea water, filtration, activated carbon adsoprtion, aeration and ozonation comprise the basic techniques for water-quality management.

There are two basic categories of aquatic culture systems: still-water and running-water (closed or open). Bacteria, unicellular plants and a variety of small invertebrates can be maintained in still water but most multicellular plants and animals require running water. Flow through (open) systems are advantageous but are not absolutely necessary as long as the seawater is filtered and water quality is maintained. King (1975) and Kinne (1976) discuss the various types of recirculating (closed) systems and their advantages for the maintenance of test organisms. Some of these advantages are that recirculating systems offer easy control and monitoring of many environmental parameters and they can be established well away from a source of seawater.

Since environmental conditions in the laboratory invariably will be different from those of the habitat from which the test organism was collected the animals should be allowed to acclimate before they are used as test organisms. Overcrowding of the test organisms should also be avoided during maintenance. Special attention should be paid to maintaining a suitable water temperature, particularly in arctic species which may have a very narrow range of temperature tolerance.

In addition to water-quality management, adequate nutrition and disease control constitute major problems in the laboratory maintenance of organisms. Maintaining filter feeders such as many pelecypod species can be accomplished without too much difficulty if abundant stocks of phytoplankton are available. Guillard (1975) describes relatively simple and reliable methods for the culture of marine phytoplankton species useful for feeding marine invertebrates. Mixtures of phytoplankton and larger zooplankton can provide adequate food supply for other species of bivalves and barnacles. However, certain groups of filter feeders cannot subsist long on plankton alone and need additional nutrients or a different diet. McCammon (1975) describes the production of a beef heart extract which has been used with great success as a supplemental food for filter feeders or other invertebrates that prefer a more solid diet, e.g., barnacles, coelenterates, pelecypods, ectoprocts, copepods and filtering worms. Specimens maintained on such a diet apparently can live for many years in a closed system.

In the successful maintenance of invertebrates fortuitously favourable bacteria, flagellates and other protozoa dominate the system. However, equilibria observed in natural habitats are seldom matched in the laboratory and if the biotic balance is upset by changes of temperature, salinity and the concentration of inorganic and organic nutrients the most troublesome members of the original biota - bacteria, fungi and protozoa - will take over and eventually kill the invertebrates. Microorganisms can be inhibited or eliminated by the use of antibiotics. D'Agostino (1975) discusses the application of various types of antibiotics in the culture of invertebrates. Streptomycin and penicillin are employed most often. Of the two, streptomycin is better suited for the culture of marine invertebrates because it is a wide spectrum antibiotic with a relatively long half-life and is not readily deactivated at alkaline pH. Natural populations of microorganisms invariably contain fungi. If bacteria are inhibited fungal growth is invariably enhanced and therefore it is probably a good idea to include a fungicide in antibiotic mixtures. Antibiotics may also be toxic to the invertebrate. It is therefore important that their effects on test organisms be known before antibiotics are used.

The laboratory maintenance of the various groups of organisms which may be used as bioassay specimens will now be discussed in general terms. Following that a more detailed discussion of the maintenance of some marine arctic species will be given.

7.4.1.1 Maintenance of Microalgae

Three of the most common microalgal species which are used as assay organisms are Phaeodactylum tricornutum, Skeletonema costatum and Dunaliella tertiolecta. These species grow in laboratory culture without great difficulty. Conventional culture methods for marine unicellular algae call for the inoculation of solid or liquid media dispensed into various types of culture containers. The more standard culture containers are limited to various size test tubes, flasks or carboys. Closures on culture containers must prevent contamination while permitting good gas exchange.

Unicellular algae must first be isolated from crude samples brought into the laboratory. Pelagic forms isolated from the natural marine habitat are obtained from plant fragments or other debris in the water, from naturally occurring blooms or from plankton tows. Benthic microalgae can be removed from sediment core samples (see Section II-5.2.2). The next step after collecting the crude sample is to obtain unialgal clone cultures and then bacteria-free cultures if possible. Ukeles (1976) defines a variety of methods for isolating representatives of single algal species and for the purification of algal species from contaminating organisms as well as media preparation, sterilization and subculturing.

Some investigators maintain that non-axenic cultures reflect a more realistic approach to studies of organisms in relation to their natural environment than do axenic cultures and that non-axenic cultures provide more characteristic information on the water mass in microalgal assay studies. However, in non-axenic cultures the results are in danger of being obscured or modified by the presence of other organisms. Purification of algal species from contaminating organisms can take place only if a culture medium is available that will satisfy all nutritional requirements and if the species undergoing purification does not harbour an endosymbiotic bacterial flora. Antibiotics alone or combined with detergents are often used to purify contaminated cultures, as a direct treatment or in conjunction with some of the other purification techniques.

Success in algal culture methodology has closely followed acquisition of information on nutritional requirements. The growth and normal biochemistry of microalgae, as in other organisms, require the availability of a varying number of mineral elements, probably 15 to 20. The nutritional requirements for microalgae also include an inorganic nitrogen source, trace metals, a carbon source, vitamins and Na, K, Mg and Ca.

Ukeles (1976) also discusses the physical aspects - illumination, temperature, salinity and pH - which are necessary for the successful culture of microalgae.

7.4.1.2 Maintenance of Ciliated Protozoa

Much of our present knowledge on the reproduction, physiology, genetics, immunology and parasitology of cells has been derived from experiments on the limnic forms such as members of the genera Tetrahymena and Paramecium. In contrast, our knowledge of organism-environment and organism-organism relationships in marine protozoans is unsatisfactory and insufficient. Very few marine ciliates have, thus far, been used as an assay organism for determining environmental quality.

Culture methods have been developed for approximately twenty species of marine ciliates. Kinne (1977) has tabulated the ciliate species which have been cultured, the media they were grown in and the food they were given. The ciliates cultured so far seem to adapt to various artificial sea waters when adjusted to the original salinity. Provasoli (1977) discusses purification of ciliate cultures by the use of various antibiotics, as well as various media which may be used for isolation and growth. Ciliate cultures can be maintained in clean and sterile Petri dishes, test tubes or various flasks. Most ciliates feed phagotrophically on bacteria but in suitable artificial media they can grow osmotrophically on dissolved nutrients.

Cristigera sp., common, bacterivorous, sediment-living marine ciliates have been used as toxicity indicators for heavy metals (Gray and Ventilla, 1973; Gray, 1974). The culture method for these ciliates can perhaps serve as a model. Sediment-living bacteria were isolated from intertidal sand and grown on ZoBell's 2216E medium marine agar. A pseudomonad was cultured under sterile conditions in a medium of seawater and 0.03% peptone as nutrient. A sand sample was taken from the sediment where the bacterium was isolated and the microfauna extracted using the seawater ice method. A flask of sterile aged seawater was inocculated with stock bacteria and incubated at 18°C for 12 hours. The mixed ciliate culture was added to the flask. One organism, Cristigera sp., grew abundantly and was isolated as a single species culture. This species was maintained for over two years on the pseudomonad diet at 6°C with routine subculturing.

7.4.1.3 Maintenance of Nematodes

Nematodes play a fundamental role in food-web relationships and as a potential food source. They inhabit a large variety of marine environments, often in high population densities and are collected in dredge or grab samples. Interest in cultivating marine or brackish nematodes is recent and adequate culture methods remain to be worked out for most representatives. The potential role of marine nematodes as indicators of water-quality and pollution load has therefore not been investigated to any great extent. Kinne (1977) has tabulated culture media and food sources for those marine and brackish nematodes which have been established in cultures. Nematodes have been grown on fungal mats in liquid culture as well as in a variety of agar media.

Nematodes live in enivronments where bacteria decompose organic matter and most utilize as food decaying or living microorganisms (bacteria, fungi) or microalgae associated with sediment habitats. Many free-living nematodes are carnivorous and feed on small metazoan animals, including other nematodes. Bacteria, yeasts, flagellates, fungi, ciliates and diatoms are among the food sources used in laboratory cultures of nematodes.

7.4.1.4 Maintenance of Polychaetes

Infaunal species of adult polychaetes should be removed from the sediment unbroken and in healthy condition. Separation from the sediment may be done in the field or back in the laboratory. A gentle stream of salt water facilitates the removal of specimens intact. It is preferable, although not always practical, to place each specimen in its own container.

Dean and Mazurkiewicz (1975) discuss in some detail the culture of polychaetes, both adults and larvae. Considerable work has been devoted to using laboratory populations of polychaetous annelids (adults and larvae) as indicators of marine pollution and water quality, in particular, the effects of heavy metals on their survival, reproduction and life cycles (Reish, 1970, 1973, 1978, 1980a, 1980b, 1984; Reish and Carr, 1978; Reish et al., 1974, 1976). Polychaetes are generally maintained in small enclosures such as Petri dishes, Boveri dishes or small aquaria. There are two basic types of culture systems: open and closed. An open sea water system, usually with a filtering system, is the most commonly used method for maintaining large numbers of adult polychaetes. The closed systems range from standing water to water kept in motion via aerators, stirrers or shakers to recirculating water systems with filters. The water should be changed frequently, especially if food is added. Since filtration systems diminish the frequency of water changes the water used in closed systems is usually filtered. Adequate temperature control is one of the most important considerations in the laboratory maintenance of polychaetes. Culturing requires a variety of non-toxic, thoroughly cleaned culture vessels; finger bowls and Petri dishes are most commonly employed.

Usually some form of substratum enhances maintenance; a layer of detritus and/or fine sediment works well. Several artificial substrates have also been used successfully; for example, absorbent cotton and the mineral, cryolite. Artificial borrows of plastic or glass tubing of appropriate diameter has been used with success to maintain nereids, arenicolids, some spionids and glycerids.

Many species of worms can be kept for extended periods of time without the addition of food, however, there is a reduction in body size. Various natural and artificial foods have been offered. The natural food include detritus, cultured phytoplankters (diatoms and flagellates), fresh or dry macroscopic algae, spinach and animals. Liver powder, powdered alfalfa and commercial fish food comprise the artificial foods which have been used.

Polychaete embryos and larvae have also been maintained in the laboratory. The larvae may be procured by the laborious process of isolating them from plankton samples or from meiobenthic samples. The most ideal method of procuring early developmental stages, however, is by inducing adults to spawn naturally in the laboratory. Temperature manipulation is the most common method of stimulating spawning. Dean and Mazurkiewicz (1975) detail the various culture systems for maintaining larval forms, both pelagic and nonpelagic, as well as appropriate food sources.

7.4.1.5 Maintenance of Echinoderms

Most echinoderms can be accommodated in aerated aquaria or tanks, often in closed sea water systems. If the animals are to be kept for any length of time (more than one week) then an open-water system is not only the best but with most species is the only way to maintain them in healthy condition (Ruggieri, 1975). Direct sunlight appears to be undesirable or even harmful to most species; dim daylight or dim artificial light, simulating natural diurnal changes, is best. Overcrowding should be avoided. Echinoderms feed on a large variety of organic materials and display a large variety of feeding types. Echinoids (sea urchins) are omnivorous opportunists which in the field feed on multicellular algae or on living or dead animals. In the laboratory, they will ingest a variety of food. They do well on a variety of sea weeds but have also been fed spinach, lettuce, meat from bivalves, annelids, crustaceans and fish, trout food, dog food, frozen shrimp and boiled egg. The food source for asteroids (starfish) depends on their feeding type. Kinne (1977) has tabulated the various food items consumed under in situ conditions by seastars. Deposit feeders (e.g., Patiria miniata) extrude their stomach over the sea bottom where they take up food items from the sediment surface. Suspension feeders (genera Henricia, Linckia, Echinaster, Porania) can be maintaned on fine particulate matter or phytoplankton. Prey seizing seastars in the field feed on molluscs, annelids, crustaceans and fish. Most prefer bivalves as a food source. In the laboratory these predatory starfish can be maintained for long periods of time on small pieces of shellfish as well as the meat of annelids, crustaceans and fish. Many other echinoderms can be maintained for several weeks without any food at all if they are kept at cold temperatures.

In the rearing of echinoderm larvae many larvae develop for a considerable period of time without food after hatching. The late pluteus stage does require food to continue development. However, very few species have had their larvae reared to the settling stage. The larvae and eggs of sea urchins have been used to assay water quality. In particular, fertilized sea urchin eggs and other developmental stages have been used as the material for a marine pollution bioassay (Kobayashi, 1971, 1972, 1973, 1977, 1980, 1981, 1984; Kobayashi and Fujinaga, 1976). Gametes of echinoderms have been obtained after natural spawning, after gonad dissection or after artificially induced spawning. Kinne (1977) gives a detailed discussion on rearing echinoderm larvae including fertilization, incubation and hatching, as well as environmental and nutritional requirements.

7.4.1.6 Maintenance of Crustaceans

(a) Copepoda

While it is easier to cultivate benthic copepods than their planktonic counterparts, planktonic copepods tend to be more sensitive to environmental changes and often are excellent water quality indicators. Culture methods for benthic and planktonic marine copepods are given in Kinne (1977). Most investigators use natural sea water, filtered and unfiltered, as the culture medium , though some researchers (Gonzalez, et al., 1975) utilize artificial sea water. A few investigators have added antibiotics and chelating agents (EDTA) to the medium. The culture containers are usually small (1 L to several litre capacity). Benthic copepods probably should have the water changed every 3-4 days. Some can survive with no food for a long period while others utilize bacteria or a variety of unicellular algae. For planktonic copepods the culture container should be slowly rotated or aerated in order to produce water movement and facilitate gaseous exchange. There should be frequent water exchange or use a recirculation or flow-through system. Avoid excess handling and too much contact between the copepods and culture-vessels walls or other hard surfaces. The primary food items of most marine copepods are unicellular algae though a few feed on animal matter. Algae which qualify as good diets for planktonic copepods include *Dunaliella* sp., *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Skeletonema costatum*. Mixed diets often give better results.

(b) Mysidacea, Isopoda, Amphipoda

These organisms have been cultured in small enclosures (beakers, finger bowls, Boveri dishes) which are frequently not aerated; in non-running, but regularly renewed water; and in larger enclosures (jars, aquaria, fibreglass tanks) in which the water is usually aerated and often recirculated and filtered. Many benthic isopods and amphipods require or prefer the provision of hiding places (e.g., plants, mollusc shells, stones) or substrata such as sand or gravel. Most forms prefer dim light or quasi darkness. Nutritional preferences vary but several food items have been used successfully. Benthic forms have been fed detritus containing bacteria, fungi or protozoans; plants such as diatoms, *Ulva lactuca*, lettuce, spinach; living animals such as oligochaetes and pieces of molluscs, crustaceans and fish. In addition, various commerical fish foods have been used successfully. Planktonic forms have been fed a variety of phytoplankton, copepods, rotifers, *Artemia salina* and organic detritus particles (Kinne, 1977).

(c) Decapoda Natantia (Shrimp and Prawns)

Adult shrimp and prawns are best kept in aquaria and related medium-sized enclosures. Decapod larvae can be accommodated in culture dishes (water renewal every second day), compartmented tray or in culture systems in running water. Many decapods are omnivorous; frequently with a tendency to favour animal food. In captivity, decapods such as shrimp and crabs accept a large variety of food items ranging from plants, invertebrates and fishes to commercial fish foods and trash foods (remains of foods used for human consumption). While a few seem to develop and grow equally well on dry (commercial fish food) and live foods, in many cases, live foods such as Artemia salina or meat of freshly killed animals (eg., clams, mussels, squid, crustaceans, fish) seem best. The diets consumed by reared decapod larvae include phytoplankton algae such as species of Dunaliella, Chlorella, Isochrysis, Skeletonema, Phaeodactylum, etc. and zooplankters such as rotifers and copepods, as well as eggs and larvae of annelids, cirripedes, molluscs, echinoderms and fishes. Multi-component (mixed) diets are more likely to meet all nutritional requirements. Benthic natantians, such as pink and brown shrimp, seek protection in sediments. Therefore, some kind of substratum or protective covering should be made available. Kinne (1977) gives a detailed discussion of rearing decapods (adults and larvae) in the laboratory.

7.4.1.7 Maintenance of Bivalve Molluscs

Juveniles and adults are best maintained in the laboratory in a flowing natural sea water system (closed or open) at controlled temperatures in trays or tanks with several inches of natural substrate (Chanley, 1975; Culliney et al., 1972). The majority of bivalves are suspension feeders; however, some are deposit feeders which obtain their food from the bottom detritus. The natural food supply in the running water is normally sufficient to maintain the organisms in good physiological condition. Supplemental feeding with phytoplankton (natural or cultured) is recommended if such a source is available. The sea water in an open sea water system can be enriched with suspensions of food algae. In a closed system a mixture of food algae can be automatically added to the tank to give a continuous, adequate level of cell density in the water. Under laboratory conditions bivalves have been raised on a variety of unicellular algae.

Since bivalve larval stages are most sensitive to environmental conditions they are perhaps better suited than the adults to be test organisms for bioassays. A few representatives have been used for toxicity tests and as experimental material for assessing the biological consequences of pollutants. More specifically, the embryos and larvae of bivalve molluscs have been used to assess the toxicity of heavy metals as well as their effects on larval survival and embryonic development (Calabrese, 1984; Calabrese and Nelson, 1974; Calabrese et al., 1973, 1977a and b). Loosanoff and Davis (1963) and Kinne(1977) contain detailed discussions of the laboratory rearing of bivalve larvae. Larvae can be obtained by screening them from plankton samples but are most often obtained from adults which are collected in the field, conditioned in the laboratory and then stimulated to spawn. Spawning can be induced by various means; thermal, electrical, chemical, etc. Thermal stimulation, the most common method, causes ripe bivalves to spawn when the temperature of the sea water is rapidly raised several degrees. The adults should be cleaned of mud and debris when brought into the laboratory to help control protozoan and bacterial contamination of gametes and young larvae. Larvae may be kept in a variety of enclosures (cylindrical in shape), including small Pyrex glass beakers, jars used in fish hatcheries for the incubation of semi-buoyant eggs, lobster jars, earthenware jars, bins and fibreglass containers (Kinne, 1977). Water quality requirements as well as optimum conditions of temperature, salinity and of larval and food concentrations must be adjusted to species specific demands and continuously controlled. Unless running water is used, the culture water should be renewed regularly (every second day). Use filtered sea water for maintaining the larvae. The food which bivalve larvae consume in situ is insufficiently known. Evidently small phytoplankton, preferably thin-walled or naked forms, constitute the most important if not the main food source. In the laboratory, no food sources other than a variety of unicellular algae have been found satisfactory for maintaining the larvae. Algae such as Isochrysis galbana, Monochrysis lutheri, Dicrateria inornata, Chlorococcum sp., Cyclotella nana, etc. have supported good growth. Though the field of diseases and parasites of larval and juvenile molluscs is fairly new, populations of bacteria and protozoans are known to cause mortality. Antibiotics, fungicides and germicidal ultraviolet rays are used to treat the sea water (Loosanoff and Davis, 1963). The main countermeasure employed for combating extensive microbial growth involves the use of antibiotics; a mixture (e.g., penicillin and streptomycin) is generally more effective than a single antibiotic.

7.4.1.8 Maintenance of Fish

Wong (1982) gives a specific description of the maintenance conditions of fish (the threespine stickleback, Gasterosteous aculeatus) used for a static bioassay procedure. In general, fish must be acclimated prior to a bioassay to determine that they are healthy and to allow them to adjust to holding conditions. Acclimation tanks and accessories must be made of non-toxic materials such as glass, porcelain, fibreglass, stainless steel, polyethylene, acrylic, polypropylene or fibreglass-reinforced polyester. These tanks should be located away from physical disturbances and preferably separated from test vessels. During acclimation a constant flow of water through the holding tank is necessary. The water should be aerated or oxygenated, cooled and filtered. The photoperiod should probably duplicate the natural one. The tank should be kept clean by periodic siphoning of settled material. To prevent overcrowding there should be at least one litre of water for every 10 grams of fish in the holding tank (Sprague, 1973). A daily inspection for unhealthy fish should be carried out. Water quality (for example, pH, salinity, temperature and dissolved oxygen) should be monitored. The holding tanks should be disinfected between batches of fish. The fish should probably be held for a 14-day acclimation period before being used; during this time they should be actively feeding. Various types of commercial fish food are available. A detailed disucssion of fish feeds can be found in Kinne (1977). Bacterial contamination and diseases can be controlled with the use of antibiotics, fungicides and by ultraviolet irradiation of the water.

Larvae of marine fishes are difficult to maintain in the laboratory; many refuse to feed and exhibit narrow, specific environmental and nutritive requirements. Kinne (1977) discusses the laboratory maintenance of larval fishes in considerable detail. The main and best food source for most marine fish larvae are zooplankters, especially copepod nauplii.

7.5 Arctic Studies

Optimal levels of major environmental factors and tolerance limits for most arctic species are not known (Percy, 1975).

Percy (1978) maintained the isopod Mesidotea entomon, both gravid females and juveniles, in recirculating sea water (Instant Ocean, 15°C, 15 ‰) at the Arctic Biological Station in Quebec The isopods were fed an excess ration of Tetramin fish food. In the Inuvik Research Laboratory Mesidotea entomon, M. sibirica and M. sabini have been held at 5°C in large (75 - 100 gallon) tanks of 30 °/oo Instant Ocean Seawater (the freshwater forms of M. entomon were held in dechlorinated tapwater). The water was continuously aerated, filtered and changed regularly. These isopods were fed twice a week with Tetramin fish food (Percy et al., 1978). In this same study some isopods were shipped to the Arctic Biological Station where they were held in a recirculating system at the same temperature and salinity. Percy (1975) has also maintained the amphipod Onisimus affinis in the Eskimo Lakes Field Laboratory and the Inuvik Research Laboratory where they were held in large tanks at a temperature and salinity approximating that of their natural habitat. Percy gives the yearly range for both salinity (bottom salinity 13.4 - 17.2 °/oo; surface salinity 9.9 - 17.2 °/oo) and temperature (bottom temperature -0.97 - +9.07°C; surface temperature -1.35 -+12.0°C) in the vicinity of the collection site. Some of these amphipods where transported south to the Arctic Biological Station where they were maintained in a high-capacity refrigerated, reciruclating salt water system at a temperature of 1° ± 1°C and a salinity of 17 ± 1 °/oo. In their study of the effects of crude oil on arctic marine invertebrates, Percy and Mullin (1975) collected eleven species (see Section 7.3.2) from the Western Beaufort Sea. All of these animals were held in a recirculating seawater system at the Arctic Biological Station's field laboratory (near the entrance to the Eskimo Lakes) at a temperature and salinity regime approximating that in the natural habitat. The salinity in the vicinity of the Eskimo Lakes collection site was about 15 - 17 °/oo and the water has an annual temperature range of about -1° to +9°C. Some of the winter collections of the amphipod Onisimus affinis were transported to Quebec and held in the recirculating sea water system (Temperature 1°C, Salinity 15 °/oo) at the Arctic Biological Station.

The NORTEC (1981) study in which static 96-hour bioassays were conducted on sixteen local organisms from the Prudhoe Bay area (see Section 7.3.2) utilized a portable laboratory which was located on the sea ice in winter and then relocated onshore prior to spring breakup. The laboratory among other equipment contained six plywood troughs with flowing sea water and drainage systems. The troughs (0.9 by 2.4 by 0.3 m) were constructed of plywood and reinforced with fibreglass. Four of these troughs were used as water baths for temperature control during bioassays and two for holding organisms. Fluorescent lighting in the laboratory remained on continuously during the study. During winter seawater was pumped into the laboratory through a polyvinyl chloride pipe placed through a hole in the sea ice. The intake was positioned approximately 1 m above the ocean floor. The hole through the sea ice was insulated and the intake pipe was heated to prevent ice formation. When the laboratory was moved onshore an intake line placed 50 m offshore in a water depth of approximately 3 m provided sea water to the laboratory. Seawater was pumped into large rubber inflatable tanks (air berm) where some settling of suspended material occurred before the water was used for cooling, holding organisms and bioassays. The water was pumped from the air berm into the laboratory or into two 4400 litre holding pools. Fish collected in the summer were placed in the two 4400 litre holding tanks. They were not fed but some food was introduced with the ambient seawater which was circulated through the pools. Pool mortalities varied from 2 to 10 percent, fluctuating with the changes in the holding tank salinity as weather and currents changed. Prior to testing, fish were transferred to a holding trough in the laboratory and held for 48 hours without food. The other bioassay organisms were apparently maintained without food in 50 litre tanks set in two cooling troughs in the laboratory. They were held for 48 hours prior to testing. The holding water was changed approximately once every six hours. Few mortalities (less than 2 percent) were observed in the handling and holding of these organisms. Characteristics of the ambient seawater during late winter were as follows: salinity varied from 32 to 34 $^{o}/oo$; temperature, -1.3°C to -1.2°C; dissolved oxygen, 10 to 12 ppm; and pH, 7.8 to 8.2. Ambient conditions measured during the summer ranged as follows: salinity, 6 to 23.5 $^{o}/oo$; temperature, 6.4°C to 12.8°C; dissolved oxygen, 9.6 to 12.2 ppm; and pH, 7.9 to 8.4.

Schneider and Hanes (1981) in a study to determine the responses of arctic benthic and epibenthic invertebrates to salinity and crude oil stress, maintained in the laboratory several local species (see Section 7.3.2) taken from near Barrow, Alaska. These animals were maintained at 32 $^{o}/oo$ salinity unless the experimental protocol demanded some other salinity. They were held at -1.0°C and in the dark in Percival constant temperature incubators under static water conditions. Holding containers were either polystyrene or polyethylene boxes and were not aerated. The oxygen content of the water was checked at intervals by Winkler titration and was found not to fall below 75 % of saturation. Animals were not fed during maintenance. Survival was excellent under these maintenance conditions and the animals appeared to be in good health for periods exceeding one month. However, laboratory maintenance of the experimental animals was usually held to no more than 2 or 3 weeks to avoid problems associated with long-term storage.

Finally, as part of the Baffin Island Oil Spill (BIOS) Project three arctic species have been collected from near Cape Hatt, N.W.T. and maintained in the laboratory for use as test organisms to investigate the behavioural responses of benthic invertebrates to chemically dispersed oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). The three test species selected were two filter-feeding bivalves, Mya truncata and Serripes groenlandicus, and a surface deposit feeder, the green sea urchin Strongylocentrotus droebachiensis. A flow-through system was constructed on-site to provide a number of 50L aquaria simultaneously with a continuous flow (approximately 100 mL/min per tank) of seawater. Water originated offshore from an 8m water depth, taken 1 m above the bottom sediment. Temperature and salinity were checked regularly and were 6±1°C and 26±3 °/oo throughout the experimental period which is similar to conditions in offshore waters where the test species were collected. Subdued natural lighting was used in the laboratory to further normalize holding conditions. Following collection the animals were transferred to the field laboratory and acclimated in the flow-through seawater system for at least 7 days prior to exposure studies. Mortalities during acclimation were less than 3%. Fresh seaweed (Laminaria sp.) and broken shell were supplied regularly to the urchins during the pre-exposure period. Serripes groenlandicus were kept in a 10 cm layer of washed and sifted coarse beach sand. Mya truncata and Strongylocentrotus droebachiensis were held without sediment in the test tanks. Holding densities did not exceed field densities and were limited to 20 individuals of medium size for each species per 50L aquarium.

7.6 Literature Cited

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7.7 Personal Communications

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APPENDIX A

DRILLING FLUIDS: ADDITIVES AND COMMON FORMULATIONS

TABLE A-1

COMMON DRILLING MUD CHEMICAL ADDITIVES

FUNCTION	COMPOUNDS
Weighting Materials	Products with high specific gravities, predominantly barite, calcite, ferrophosphate ores, siderite, and iron oxides (hematite), are used to increase drilling mud weight.
Gelling Agents	Bentonite clay most common; attapulgite or sepiolite clays for high formation brine concentration.
Thinners and Dispersants	Lignosulfonates, tannins, lignite and various polyphosphates such as sodium acid pyrophosphate (SAAP); act by deflocculating associated clay particles.
Alkalinity and pH Control Agents	Caustic soda, sodium carbonate, sodium bicarbonate,and lime commonly used to control the alkalinity of the drilling fluid and secondarily to control bacterial growth.
Pipe Release Agents	Surfactants and detergents in mineral oil, straight diesel oil systems for specialized purposes such as freeing a stuck pipe.
Filtrate Loss Reducers and Viscosifiers	Bentonite clays, a range of cellulose polymers such as sodium carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC), and pre-gelled starch, xanthum and guar gum polymers, added to drilling fluid to prevent loss of the liquid phase into the formation.
Lost Circulation Materials	Wood chips or fibres, mica, sawdust, leather, nut shells, cellophane, shredded rubber, fibrous mineral wool, and perlite all used to plug pores in the well-bore wall and to reduce or stop fluid loss into the formation.
Shale Control Inhibitors	Gypsum, sodium silicate, polymers, limes, potassium chloride and salt reduce wall collapse caused by swelling or hydrous distintegration of shales.
Flocculants	Salt (or brine), hydrated lime, gypsum, and sodium tetraphosphate cause suspended colloids to flocculate.

TABLE A-1 (continued)

COMMON DRILLING AND CHEMICAL ADDITIVES

FUNCTION	COMPOUNDS
Scale Inhibitors	Caustic soda, soda ash, sodium bicarbonate, and certain polyphosphates added to control the calcium buildup which prevents proper functioning of drilling equipment.
Surface Active Agents (Surfactants)	Emulsifiers, demulsifiers, and flocculants reduce the relationship between viscosity and solids concentration, vary the gel strength, and reduce the fluid plastic viscosity.
Foaming Agents	Surfactants and detergents, such as ethoxylated alkyl phenols and organic sulphonates, designed to foam in the presence of water and allow air or gas release when drilling through formations producing water.
Defoamers	Aluminum stearate, sodium aryl sulfonate, tributyl phosphate, alcohols designed to reduce foaming action, particularly in brackish waters and saturated saltwater muds.
Emulsifiers	Ethyl hexanol, silicone compounds, modified lignosulfonates, and anionic and non-ionic products are used as emulsifiers to create an homogeneous mixture of two liquids.
Corrosion Inhibitors	Hydrated lime and amine salts added to drilling fluids to reduce corrosion.
Lubricants	Certain hydrocarbons, mineral and vegetable oils, graphite powder, and soaps used as lubricants to reduce friction between the drill bit and the formation.
Biocides	Paraformaldehyde, gluteraldehyde, dithiocarbamates, alkylamines, quaternary amines, caustic soda, lime, and starch preservatives typically used to reduce the bacteria count in the mud system. Halogenated phenols are no longer permitted for offshore use in the U.S.
Oxygen Scavenger	Sodium, ammonium bisulphite

TABLE A-1 (continued)

COMMON DRILLING AND CHEMICAL ADDITIVES

FUNCTION	COMPOUNDS
H_2S Scavenger	Sodium dichromate; zinc carbonate, sulfonate, chromate
Drill Pipe/Collar Dope	Copper, lead, zinc.
Permafrost well-bore Temperature Depressant	Potassium chloride; relatively high use in Canadian Arctic.

TABLE A-2

TYPICAL OIL-BASE MUD FORMULATION AND PROPERTIES

Properties(1)	
Density	1.5 g/cm^3 (12.5 lb/gal)
Percent oil (vol.)	58
Percent water (vol.)	20
Percent solids (vol.)	22
Oil:Water Ratio	76:24

Composition(2)	Weight Percent	Typical Constituents
Diesel	30	-
Water (sea water)	13	-
Barite	39	Barium sulphate
Calcium chloride	7	-
Oil stabilizer	3.6	Asphaltene
Emulsifier	0.8	Ethoxylated tall oil; resin polyamine
Filtration control agent	3.2	Starch, cellulose
Lime	2.0	-
Clay	1.2	Organophilic (quaternary amine) bentonite

TABLE A-3

COMPOSITION OF A TYPICAL PARAFFIN-BASE MUD

		CONCENTRATION
Mud Weight		1.2 g/cm^3 (10 lb/U S gal)
Low-aromatic oil (paraffin)		30% (by volume)
Water		29% (by volume)
Barite		27% (by volume)
Sodium chloride		12% (by volume)
Lime		2% (by volume)
Clay	- (Organophilic clay)	3% (by volume)
Emulsifier	- (Fatty acid amide)	
Dispersant and oil wetting agent	- (Modified imidazoline)	
High temperature stabilizer	- (Gilsonite (asphaltene))	
Dispersant	- (Calcium sulphonate)	
Emulsifier component	- (Calcium oxide)	

Note: Rounding error results in total = 103 percent.

TABLE A-4

COMMON DRILLING FLUID TYPES - UNITED STATES OFFSHORE

DRILLING FLUID TYPE	MAJOR COMPONENTS	USE RATE IN WHOLE MUD	
		(kg/m^3)	(lb/bbl)
Spud Mud	Attapulgite/bentonite	30 - 140	10 - 50
	Barite	0 - 140	0 - 50
	Lime	1 - 2	0.5 - 1.0
	Caustic (NaOH)	0 - 6	0 - 2
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Sea water	as needed	
Saltwater/KCl/ Polymer Mud	Barite	0 - 1,280	0 - 450
	Drilled solids	60 - 280	20 - 100
	KCl	15 - 140	5 - 50
	Starch	6 - 35	2 - 12
	Cellulose polymer	0.7 - 15	0.25 - 5
	Caustic (NaOH)	1 - 9	0.5 - 3
	Xanthum gum polymer	0.7 - 6	0.25 - 2
	Sea water	as needed	
Seawater Gel Mud	Drilled solids	60 - 280	20 - 100
	Barite	0 - 140	0 - 50
	Attapulgite/Bentonite	30 - 140	10 - 50
	Caustic (NaOH)	1 - 9	0.5 - 3
	Cellulose polymer	0 - 6	0 - 2
	Lime	0 - 6	0 - 2
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Sea water	as needed	
Seawater Lignofonate Mud	Barite	70 - 1,280	25 - 450
	Drilled solids	60 - 280	20 - 100
	Attapulgite/Bentonite	30 - 140	10 - 50
	Lignosulfonate	6 - 40	2 - 15
	Lignite	3 - 30	1 - 10
	Caustic (NaOH)	3 - 15	1 - 5
	Cellulose polymer	0.7 - 15	0.25 - 5
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Sea water	as needed	

TABLE A-4 (continued)

COMMON DRILLING FLUID TYPES - UNITED STATES OFFSHORE

DRILLING FLUID TYPE	MAJOR COMPONENTS	USE RATE IN WHOLE MUD (kg/m^3)	USE RATE IN WHOLE MUD (lb/bbl)
Lightly Treated Lignosulfonate Freshwater/ Seawater Mud	Barite	0 - 500	0 - 180
	Drilled solids	60 - 280	20 - 100
	Bentonite	30 - 140	10 - 50
	Lignosulfonate	3 - 17	2 - 6
	Lignite	0 - 11	0 - 4
	Caustic (NaOH)	3 - 9	1 - 3
	Cellulose polymer	0 - 6	0 - 2
	Lime	0 - 6	0 - 2
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Sea water	as needed	
Lignosulfonate Freshwater Mud	Barite	0 - 1,280	0 - 450
	Drilled solids	60 - 280	20 - 100
	Bentonite	30 - 140	10 - 50
	Lignosulfonate	11 - 42	4 - 15
	Lignite	6 - 30	2 - 10
	Caustic (NaOH)	6 - 15	2 - 5
	Cellulose polymer	0 - 6	0 - 2
	Lime	0 - 6	0 - 2
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Defoamer/detergents	0	0
	Freshwater	as needed	
Lime Mud	Barite	70 - 500	25 - 180
	Drilled solids	60 - 280	20 - 100
	Bentonite	30 - 140	10 - 50
	Lime	6 - 60	2 - 20
	Lignosulfonate	6 - 40	2 - 15
	Lignite	0 - 30	0 - 10
	Caustic (NaOH)	3 - 15	1 - 5
	Soda ash/sodium bicarbonate	0 - 6	0 - 2
	Freshwater	as needed	
Nondispersed Mud	Barite	70 - 500	25 - 180
	Drilled solids	60 - 200	20 - 70
	Bentonite	15 - 40	5 - 15
	Acrylic program*	1 - 6	0.5 - 2
	Freshwater	as needed	

* polyacrylates and polyacrylamides

APPENDIX B

PARTIAL LIST OF NORTH AMERICAN MANUFACTURERS OF CORERS AND GRAB SAMPLERS

1. Kahl Scientific Instrument Corp.
   P.O. Box 1166
   El Cajon, California 92022
   (all types of corers and grab samplers)

2. Benthos Inc.
   North Falmouth, Massachusetts 02556
   (gravity, piston and free-fall corers)

3. Wildlife Supply Co.
   301 Cass Street
   Saginau, Michigan 48602
   (gravity corers, Eckman and Ponar grabs)

4. Hydro-Products
   11777 Sorrento Valley Road
   San Diego, California 92121
   (grabs, gravity corers)

5. TSK. Co. Ltd.
   15109 30th Ave. S.E.
   Bothell, Washington 98011
   (grabs, gravity corers)

6. Horizon Ecology Company
   7435 North Oak Park Ave.
   Chicago, Illinois 60648
   (small grabs, gravity corers)

7. Intersea Research Corporation
   Post Office Box 2389
   La Jolla, California 92037
   (gravity corers, grabs)

8. Ocean Instruments
   5312 Banks Street
   San Diego, California 92110
   (box corers, Soutar-Van Veen grab)

9. M.S.E.
   Downsview, Ontario
   (416) 661-5646

10. T. Thompson Ltd.
    Vancouver, B.C.
    (604) 926-3201

APPENDIX C

PARTIAL LIST OF AIR CARRIERS
SERVING MAJOR ARCTIC COMMUNITIES

(from 1984 Directory of Canadian Commercial Air Services,
Canadian Transport Commission, Ottawa)
(phone 819-997-6030 for additional detail)

CLASSIFICATION OF AIR SERVICES

(a) Class 1: Scheduled commercial air service, being a service that is operated wholly within Canada and that is required to provide public transportation of persons, goods or mail by aircraft, serving points in accordance with a service schedule at a toll per unit of traffic;

(b) Class 2: Regular Specific Point commercial air service, being a service that is operated wholly within Canada and that is required to provide, to the extent that facilities are available, public transportation of persons, goods or mail by aircraft, serving points in accordance with a service pattern at a toll per unit of traffic;

(c) Class 3: Specific Point commercial air service, being a service that is operated wholly within Canada and that offers public transportation of persons, goods or mail by aircraft, serving points consistent with traffic requirements and operating conditions at a toll per unit of traffic;

(d) Class 4: Charter commercial air service, being a service that is operated wholly within Canada and that offers public transportaion, on reasonable demand, of persons or goods from the base specified or the protected base denoted in the licence issued for that commercial air service at a toll per mile or per hour for the charter of an entire aircraft, or at such other tolls as may be allowed by the Committee, and includes recreational flying;

(e) Class 5: Contract commercial air service, being a service that is operated wholly within Canada from the base specified in the licence issued for that commercial air service, that offers transportation of persons or goods solely under contracts of carriage with users with whom the air carrier has a substantial relationship through corporate structure of financial control and that does not hold out to the general public, or a class or segment thereof, the offer of transportation by air;

(f) Class 6: Flying Club commercial air service, being a service that is operated wholly within Canada from the base specified in the licence issued for that commercial air service and that provides flying training and recreational flying to members of a flying club incorporated as a non-profit organization;

(g) Class 7: Specialty commercial air service, being a service that is operated from the base specified in the licence issued for that commercial air service for any purpose not provided for by any other class of service and, without limiting the generality of the foregoing, for any of the following purposes:

(i) "aerial application and distribution", - (AAD) - being the application of chemicals or distribution of other materials from aircraft to

(A) inhibit and destroy insect life and other forms of organism injurious to plants, crops and forests, or

(B) foster the growth of crops, forests or fish, including agricultural flying, aerial pest control, spraying, seeding and reseeding, forest cultivation and fish cultivation;

(ii) "aerial construction", - (ACON) - bing the use of rotating wing aircraft in construction work, including aerial hoisting, mountain tram line construction, aerial pole setting and aerial power line construction;

(iii) "aerial control", - (AC) - being fire suppression, fire or frost prevention or altering the normal processes of weather, including aerial fire control, forest fire protection, firefighting, forest firefighting, forest protection, water pumping, forest control, hail supression, aerial frost control, rain making, fog making, fog dispersal and cloud seeding;

(iv) "aerial inspection, reconnaissance and advertising", - (AIRA) - being

(A) the reporting from aerial observation upon events, natural phenomena related to man-made objects, or

(B) the providing of visual messages in the atmosphere, including aerial patrol and inspection, ice reconnaissance, seal spotting, forest inspection and administration, forest patrol, pipeline patrol, powerline patrol, news service and aerial advertising;

(v) "aerial photography and survey", - (APS) - being

(A) the taking of photographs or the recording in other tangible form of phenomena on, under or above the earth's crust by a carrier using a camera or other measuring or recording device mounted in or attached to the carrier's aircraft and under the carrier's control, and

(B) the eventual delivery of the photograph or other record to the client in finished, semi-finished or other tangible form, including aerial photography, scintillometer survey, aerial prospecting and geophysical survey;

(vi) "aerial photography restricted to scenics", - (AP) - being the recording of scenes only and not involving any interpretive services or the creation of maps of any kind;

(vii) "flying training", - (FT) - being an air service for the purpose of instructing a person in the art and science of pilotage and operation and navigation of aricraft; and

(viii) "recreational flying", - (RF) - being flights that originate and terminate at the same place without landing at any other place for the purpose of taking on or discharging passengers and that are

(A) flown over a standard course that has been advertised by the carrier

(B) conducted for the sole purpose of the recreation of the passengers, and

(C) charged for at a rate per seat per unit of time, including sightseeing, barn-storming and parachute jumping;

(h) Class 8: International Scheduled commercial air service, being a service that is operated between points in Canada and points in any other country and that is required to provide public transportation of persons, goods or mail by aircraft, serving such points in accordance with a service at a toll per unit of traffic;

(h) Class 8: Interntional Scheduled commercial air service, being a service that is operated between points in Canada and points in any other country and that is required to provide public transportation of persons, goods or mail by aircraft, serving such points in accordance with a service schedule at a toll per unit of traffic;

(i) Class 9-2: International Regular Specific Point commercial air service, being a service that is operated between points in Canada and points in any other country and that is required to provide, to the extent that facilities are available, public transportation of persons, goods or mail by aircraft, serving such points in accordance with a service pattern at a toll per unit of traffic;

(j) Class 9-3: International Specific Point commercial air service, being a service that is operated between points in Canada and points in any other country and that offers public transportation of persons, goods or mail by aircraft, serving such points consistent with traffic requirements and operating conditions at a toll per unit of traffic;

(k) Class 9-4: International Charter commercial air service, being a service that is operated between Canada and any other country and that offers public transportation, on reasonable demand, of persons or goods at a toll per mile or per hour for the charter of the entire aircraft, or at a toll on such other basis as may be allowed by the Committee; and

(l) Class 9-5: International Contract commercial air service, being a service that is operated between Canada and any other country from the base specified in the licence issued for that commercial air service, that offers public transportation of persons or goods solely under contracts of carriage with users with whom the air carrier has a substantial relationship through corporate structure or financial control and that does not hold out to the general public, or a class or segment thereof, the offer of transportation by air.

AIRCRAFT GROUPS ACCORDING TO WEIGHT

The groups for commercial air services based on the weight of the aricraft used in the operation of the service are as follows:

(a) commercial air services operated with fixed wing aircraft,

  (i) Group A, having a maximum authorized take-off weight on wheels not greater than 4 300 pounds,
  (ii) Group B, having a maximum authorized take-off weight on wheels greater than 4 300 pound, but not greater than 7 000 pounds,
  (iii) Group C, having a maximum authorized take-off weight on wheels greater than 7 000 pounds, but not greater than 18 000 pounds,
  (iv) Group D, having a maximum authorized take-off weight on wheels greater than 18 000 pounds, but not greater than 35 000 pounds,
  v) Group E, having a maximum authorized take-off weight on wheels greater than 35 000 but not greater than 75 000 pounds,
  (vi) Group F, having a maximum authorized take-off weight on wheels greater than 75 000 pounds, but not greater than 150 000 pounds,
  (vii) Group G, having a maximum authorized take-off weight on wheels greater than 150 000 pounds, but not greater than 350 000 pounds, and
  (viii) Group H, having a maximum authorized take-off weight on wheels greater than 350 000 pounds, and

(b) commercial air services operated with rotating wing aircraft,

  (i) Group A-RW, having a maximum authorized take-off weight not greater than 4 409 pounds,
  (ii) Group B-RW, having a maximum authorized take-off weight greater than 4 409 pounds, but not greater than 7 500 pounds,
  (iii) Group C-RW, having a maximum authorized take-off weight greater than 7 500 pounds, but not greater than 18 000 pounds,
  (iv) Group D-RW, having a maximum authorized take-off weight greater than 18 000 pounds, but not greater than 35 000 pounds,

(v) Group E-RW, having a maximum authorized take-off weight greater than 35 000 pounds but not greater than 75 000 pounds

(vi) Group F-RW, having a maximum authorized take-off weight greater than 75 000 pounds.

EXPLANATION OF ABBREVIATIONS

AAM - Aerial Ambulance
AAD - Aerial Application and Distribution
AC - Aerial Control
ACON - Aerial Construction
AIRA - Aerial Inspection, Reconnaissance and Advertising
AP - Aerial Photography restricted toScenics
APS - Aerial Photography and Survey
FT - Flying Training
RF - Recreational Flying
CP - Crop Pollination
CSO - Cedar Salvage Operations
C - Charter/Specialty (Fixed Wing Aircraft)
H - Charter/Specialty (Rotating Wing Aircraft)
S - Scheduled (Fixed Wing and Rotating Wing Aircraft)
NS - Non-Scheduled (Fixed Wing and Rotating Wing Aircraft)

Air Carrier	Class	Aircraft Group	License
Arctic Bay, N.W.T.			
Kenn Borek Air Ltd.	3	ABC*D	NS
	See Resolute Bay, *DC-3		
First Air	3	CD	NS
	See Frobisher Bay, Group C to serve only Repulse Bay		
Cambridge Bay, N.W.T.			
Northwest Territorial Airways	2	+D+F	NS
	See Yellowknife + Groups D + F restricted		
	3	*D*F	+NS
	* Restricted + Suspended in part		
Pacific Western Airlines Ltd.	2	FG	NS
	See Edmonton (Alta.)		
Simpson Air (1981) Limited	4	AB	C
Cape Dorset, N.W.T.			
Austin Airways Limited	2	BCDE	+* NS
	See Timmins (Ont.) * Restricted + No Local Traffic between La Grande 2 + Great-Whale-River		
First Air	3	CD	NS
	See Frobisher Bay (N.W.T.) Group C to serve Repluse Bay		
	2	CDE	NS
Cape Dyer, N.W.T.			
Nordair Ltd.	2	EF	* NS
	See Montreal (Que.) * Restricted		
Cape Parry, N.W.T.			
Aklavik Flying Service Limited	See Aklavik3(N.W.T.)	BC	NS

Air Carrier	Class	Aircraft Group	License
Coppermine, N.W.T.			
Northwest Territorial Airways Ltd.	2	+D+F	NS
	See Yellowknife (N.W.T.) +Group D+F Limited		
	3	*D*F	+NS
	*Restricted (Purposely omitted) +Suspended in part		
Coral Harbour, N.W.T.			
Calm Air	3	CE	NS
	See Rankin Inlet (N.W.T.)		
	3	E	NS
	See Churchill (Man.)		
	2	E	NS
	See Churchill (Man.)		
First Air	3	CD	NS
	See Frobisher Bay (N.W.T.) Group (to service Repulse Bay only)		
Eureka, N.W.T.			
Bradley Air Services Limited	4	BCD	C
Eskimo Point, N.W.T.			
Calm Air	2	E	NS
	See Churchill (Man.)		
	3	CE	NS
	See Rankin Inlet (N.W.T.)		
Fort Chimo, Que.			
Airgave Ltd.	3	ABCD	NS
	See Schefferville (Que.)		
Austin Airways Limited	2	BCDE	+*NS
	See Timmins (Ont.) *Restricted +No local traffic between La Grande 2 and Great-Whale-River		

Air Carrier	Class	Aircraft Group	License
Fort Chimo, cont'd.			
Johnny May's Air Charters Ltd.	4	b	C
Nordair Ltd.	2	EF	*NS
	See Montreal (Que.) *Restricted		
	2	EF	*(NS)
	See Montreal (Uqe.) *Restricted		
Frobisher Bay, N.W.T.			
Bradley Air Service Limited	4	CDE	C9-
	4	CDE	CF
First Air	2	CDE	NS
	Serving Hall Beach, Cape Dorset, Clyde River, Pangnirtung, Broughton Island, Pond Inlet, Nanisivik (N.W.T.)		
	3	C	NS
	Serving Lake Harbour (N.W.T.), Wakeham (Que.)		
	3	CD	NS
	Serving Broughton Is., Pangnirtung, Cape Dyer, Cape Dorset, Coral Harbour, Clyde River (Cape Christian), Igloolik, Hall Beach, Pelly Bay, Repluse Bay, Arctic Bay/Nanisvik, Pond Inlet (N.W.T. Group C a/c to serve Repluse Bay		
	9-3	*E	CF
	Serving Nuuk (Godithaab), Greenland. *Restricted to propeller driven aircraft		
Greenland Air Inc.	9-3	+E	
	Serving Godithaab (Nuuk), Greenland *Restricted to propeller driven aircraft		

Air Carrier	Class	Aircraft Group	License
Frobisher Bay, N.W.T., cont'd.			
Nordair Ltd.	2	EF	*NS
	See Montreal (Que.) *Restricted		
	9-3	EFG	CF
	Serving Sandre, Stromfjord, Greenland, Denmark		
	4	EFG	C
	2	EF	*NS
	See Toronto (Ont.) *Restricted		
Northwest Territorial Airways Ltd.	2*F	NS	
	See Yellowknife *Restricted (Purposely omitted)		
Gjoa Haven, N.W.T.			
Northwest Territorial Airways Ltd.	3	*D*F	+NS
	See Yellowknife *Restricted +Suspended in port		
Grise Fiord, N.W.T.			
Kenn Borek Air Ltd.	3	ABC*D	NS
	See Resolute Bay (N.W.T.) *DC-3 aircraft		
Hall Beach, N.W.T.			
Bradley Air Service Limited	4	C	C
First Air	2	CDE	NS
	See Frobisher Bay		
	3	CD	NS
	See Frobisher Bay Group C to serve only Repulse Bay		
Nordair Ltd.	2	EF	*NS
	See Montreal (Que.) *Restricted		

Air Carrier	Class	Aircraft Group	License
Holman Island, N.W.T.			
Northwest Territorial Airways Ltd.	2	+D+F	NS
	See Yellowknife (N.W.T.) *Restricted		
	3	+D*F	+NS
	See Yellowknife (N.W.T.) *Restricted (purposely omitted) +Suspended in port		
Igloolik, N.W.T.			
First Air	3	CD	NS
	See Frobisher Bay (N.W.T.) Group C to serve Repulse Bay only		
Inuvik			
Alaska Air Ltd.	4	AB	C
	9-4	AB	CF
Aklavik Flying Service Limited	4	BC	C
	9-4	BC	CF
	7AIRA	B	C
	3	BC	NS
	Serving Fort McPherson, Aklavik, Tuktoyaktuk, Sachs Harbour, Cape Parry, Paulatuk (N.W.T.)		
Antler Avaition Ltd.	4	A	C
	9-4	A	CF
	7AC	A	C
	7	A	C
Inuvik Coastal Airways Ltd.	4	C	C
	9-4	C	CF
Kenn Borek Air Ltd.	4	CS	C
	9-4	CD	CF
	2	CD	NS
	Serving Aklavik, Fort McPherson, Tuktoyaktuk, Sachs Harbour (N.W.T.)		
	3	CD	NS
	Serving Aklavik, Fort McPherson, Tuktoyaktuk, Sachs Harbour, Paulatuk (N.W.T.)		

Air Carrier	Class	Aircraft Group	License
Inuvik, cont'd.			
Shirley Air Services Ltd.	4 * Suspended	A	* H
Trans North Air	2 See Whitehorse (N.W.T.) * Restricted	BCD	* NS
EUREKA, N.W.T.			
Bradley Air Services Limited	4	BCD	C
Eskimo Point, N.W.T.			
Calm Air	2 See Churchill (Man.)	E	NS
	3 See Rankin Inlet (N.W.T.)	CE	NS
Pangnirtung, N.W.T.			
First Air	3 See Frobisher Bay (N.W.T.) Group C to serve Repulse Bay	CD	NS
	2 See Frobisher Bay (N.W.T.)	CDE	NS
Pond Inlet, N.W.T.			
First Air	2 See Frobisher Bay (N.W.T.)	CDE	NS
	3 See Frobisher Bay (N.W.T.)	CD	NS
Kenn Borek Air Ltd.	3 See Resolute Bay (N.W.T.) * DC-3 aircraft	ABC*D	NS

Air Carrier	Class	Aircraft Group	License
Rankin Inlet, N.W.T.			
Calm Air	3	CE	NS
	Serving Chesterfield Inlet, Whale Cove, Repulse Bay, Baker Lake, Coral Harbour, Eskimo Point, Cullaton Lake (N.W.T.): Churchill, Lynn Lake, Thompson (Man.)		
	3	E	NS
	See Churchill (Man.)		
	2	E	NS
	See Churchill (Man.)		
	4	C*E	C
	*Open Point		
Keewatin Air Ltd.	4	AB*C	C
	7AC	AB*C	C
	7AIRA	AB*C	C
	7AP	AB*C	C
	* Limited to aircraft having a maximum authorized take-off weight on wheels of not greater than 11,000 pounds.		
Northwest Territorial Airways Ltd.	2	*F	NS
	See Yellowknife		
	* Restricted (Purposely omitted)		
Repulse Bay, (N.W.T.)			
Calm Air	3	CE	NS
	See Rankin Inlet (N.W.T.)		
First Air	3	CD	NS
	See Frobisher Bay (N.W.T.) Group C to serve Repulse Bay		

Air Carrier	Class	Aircraft Group	License
Resolute Bay, N.W.T.			
Bradley Air Services Limited	4	+ABCD*E	C
	* Restricted to one-propeller driven type aircraft in Group E; + Suspended		
Kenn Borek Air Ltd.	3	ABC*D	NS
	Serving Arctic Bay, Grise Fiord, Pond Inlet, Nanisivik (N.W.T.)		
	4	BC*D	C
	9-4	BC*D	CF
	7AP	BC*D	C
	7APS	BC*D	C
	* Limited to DC-3 in Group D only		
Nordair Ltd.	2	EF	* NS
	See Montreal (Que.) * Restricted		
Pacific Western Airlines Ltd.	2	FG	NS
	See Edmonton (Alta.)		
Sachs Harbour, N.W.T.			
Aklavik Flying Service Limited	3	BC	NS
	See Inuvik (N.W.T.)		
Kenn Borek Air Ltd.	2	CD	NS
	See Inuvik (N.W.T.)		
	3	CD	NS
	See Inuvik (N.W.T.)		
Spence Bay, N.W.T.			
Northwest Territorial Airways Ltd.	3	*D*F	+ (NS)
	See Yellowknife (N.W.T.) * Restricted + Suspended in port		

Air Carrier	Class	Aircraft Group	.icense
Tuktoyaktuk, N.W.T.			
Aklavik Flying Service Limited	3 See Inuvik (N.W.T.)	BC	NS
Kenn Borek Air Ltd.	2 See Inuvik (N.W.T.)	CD	NS
	3 See Inuvik (N.W.T.)	CD	NS
Ram Air Charter Limited	3 See Fort McPherson (N.W.T.)	ABC	NS

APPENDIX D

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APPENDIX E

A worked example for ordination by reciprocal averaging

APPENDIX E

A worked example of ordination by reciprocal averaging (reproduced verbatim from Hill, 1973; for additional information consult Hill)

	(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)	(R)	(1)	(2)	(2a)	(3)
(i)	1	0	0	1	1	0	0	1	4	100	52.5	55	44.3
(ii)	0	1	1	0	0	1	0	1	4	0	37.5	0	36.2
(iii)	1	1	0	0	0	1	1	0	4	100	65.0	100	63.4
(iv)	1	1	1	1	1	0	0	1	6	0	43.3	21	39.3
(v)	1	1	0	1	0	0	0	1	4	100	56.7	70	47.2
(vi)	1	0	0	0	1	0	0	0	2	0	46.7	33	46.0
(C)	5	4	2	3	3	2	1	4	24				
(1)	60.0	50.0	0.0	66.7	33.3	50.0	100.0	50.0					
(2)	55.8	47.8	10.5	48.7	36.3	50.0	100.0	36.5					

……

(11)	31.8	50.5	48.4	19.7	10.0	86.0	100.0	32.7
(11a)	24	52	42	11	0	8.4	100	25

The calculations are represented schematically in the foregoing table. The datamatrix is given in the top left-hand corner, and (R) and (C) are the row (species) and column (stand) totals respectively. Column (1) is an arbitrarily chosen set of starting scores. In practice these should be chosen to reflect what is suspected of being the main gradient. A good choice will much reduce the amount of calculation required.

Row (1) is derived from column (1) by averaging. Thus the entry in row (1) column (v) is 33.3, being the average of 100, 0 and 0, which are the scores in column (1) corresponding to the non-zero entries of column (v). Column (2) is defined similarly. Thus the entry in column (2) row (i) is the average of 60.0, 66.7, 33.3 and 50.0 - these being the scores in row (1) corresponding to the non-zero entries of row (i). Column (2a) is derived from column (2) by rescaling, and is given by the formula:

column (2a) = 100 x (column (2) - 37.5)/27.5.

This ensures that the range of column (2a) is 0 to 100, since 27.5 is the range of column (2) and 37.5 is its minimum value. By continuing in this manner, the following sequence of species (row) scores is obtained.

(1)	(2a)	(3a)	(4a)	(5a)	(6a)	(7a)	(8a)	(9a)	(10a)	(11a)	(12a)	(12)
100	55	30	8	0	0	0	2	3	4	5	5	23.5
0	0	0	6	23	40	52	60	66	70	72	73	55.9
100	100	100	100	100	100	100	100	100	100	100	100	68.6
0	21	11	0	3	10	14	18	21	23	25	26	33.2
100	70	40	18	12	16	19	24	26	28	29	30	35.1
0	33	36	26	16	10	5	0	0	0	0	0	20.9

It takes eleven iterations to reach stability of the scores, but this is the result of making a bad initial choice. Three or four iterations should normally suffice if a good initial choice is made. The final stand (column) scores are derived by rescaling row (11) to form row (11a) as indicated in the original table. The eigenvalue (latent root) corresponding to the first axis is a measure of how much the range of the scores contracts in one iteration. The range of column (12) (shown after column (12a)) is 47.7, and it is derived from column (11a) which has a range of 100. Hence the estimate of the eigenvalue is 0.477. These calculations should be done with the data on one piece of quadrille paper and the scores on another, matching the two side by side.

When the first axis has been obtained, the second is considered. A good starting point for the scores of the second axis is obtained by using a set of scores which were fairly near to the final ones for the first axis. In this case column (8a) is used. Before iteration, these scores have to be adjusted by subtracting a multiple of the final first axis. This multiple is estimated as follows.

z	R	Rz	$R\bar{z}$	x	y	(13)	(13a)	(14a)	(15a)
5	4	20	165	- 145	2	- 3.0	71	62	59
73	4	292	165	127	60	- 12.4	0	0	0
100	4	400	165	235	100	0.8	100	94	89
26	6	156	247	- 91	18	- 7.8	35	34	33
30	4	120	165	- 45	24	- 5.8	50	45	41
0	2	0	82	- 82	0	0 94	100	100	
	24	988		- 1					

The column z is the first axis; R is the row totals and y is the set of scores to be adjusted (in this case equal to column (8a)). Multiply R by z to form Rz. Form $\bar{z}$ a weighted mean value of z by taking $\bar{z} = \Sigma Rz/\Sigma R$.

In this case,

$$\bar{z} = 988/24 = 41.17.$$

Form a column $R\bar{z}$ by multiplying R by $\bar{z}$; then subtract $R\bar{z}$ to derive $x = Rz - R\bar{z}$. (A check at this point is that, apart from round-off error, x should sum to zero.) The multiple of z to be subtracted from y is given by

$$\Sigma xy/\Sigma xz,$$

which in this case is 0.992. Column (13) is therefore y - 0.992z, and after rescaling to derive column (13a) the iterations are continued in the usual way. The first axis will slowly re-establish itself if the appropriate multiple of z (i.e., xy'/ xz) is not at intervals subtracted from subsequent scores y'; but this need not be done very often. The column (15a) derived after two iterations from (13a) has not been further corrected for the first axis, but it may nonetheless be taken as a reasonable estimate of the second. The estimate of the second eigenvalue, derived from column (15) (not shown), is 0.305.

These calculations are rather laborious. They would be worth the trouble if a good ordination were required in the absence of a computer.

APPENDIX F

BIOLOGICAL ASSAYS

1. INTRODUCTION

A bioassay generally involves the exposure of organisms in the laboratory to various substances in an attempt to predict whether the disposal of those substances in the environment will cause adverse effects. "Bioassay" has been defined in several ways:

(1) "a test in which the quality or strength of a material is determined by the reaction of a living organism to it" (Sprague, 1973);

(2) "a procedure in which the responses of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combination (APHA, AWWA, WPCF, 1980);

(3) a test estimating "the amount of biologically active substances by the level of their effects on organisms" (Chapman and Long, 1983).

Although the methodology of bioassays appears to be simple, in actual practice it is not since many variations often occur due to specific requirements of the investigator, the equipment that is available, the nature of the test material and the organisms available as the test species. Consequently, the specific goals of bioassays as well as equipment limitations influence the type of test that can be run.

Although the term "bioassay" is often used interchangeably with the term "toxicity testing" there is an important distinction; the goal of toxicity testing is to measure the adverse (i.e., toxic) effects of a substance on organisms whereas the goal of a bioassay is to determine the response of an organism, whether stimulatory or inhibitory, to a substance (Craddock, 1977; Stephan, 1977). The two terms are used interchangeably in the former sense in this review. In addition, this review restricts itself to a discussion of whole organism bioassays and does not consider the use of bioassays in genotoxicity studies.

Although the primary objective of a bioassay is to predict what might occur in the environment (Buikema et al., 1982), the predicative capability of bioassay test data for environmental impact has proven often to be poor (Kamlet, 1983; Monk, 1983). This will improve when there is a large data set of intercomparible results achieved by standardizing such variables as treatment of the test organism, preparation of the toxicant and by providing pertinent data and results (Craddock, 1977). Significant correlations between toxicity as determined by bioassays and the ecological quality of the environment are rare. There is a concensus of the general requirements of how tests should be conducted to help make such predictions but there is no single definitive method. On the contrary, there are many variations on the overall theme of exposing various types of organisms to various concentrations of material for varying lengths of time depending upon the specific goal of the investigator.

Despite the difficulty in extrapolating results from bioassays to the environment, a bioassay is the only method available for assessing the potential toxic effects of substances on indigeneous organisms and as such is a powerful screening test. Data gathered from field surveys are mainly descriptive and after the fact. Data from chemical tests, even elaborate and sophisticated ones, cannot be used to predict adverse (i.e., toxic) effects of a substance on organisms. It is impractical to analyse for every possible chemical in most wastes or environmental material, and to predict their interaction with other materials in the environment or with the organisms or both (Jennet et al., 1980; Cairns and Schalie, 1980). As Engler (1980) stated, "there are no solid-phase chemical analyses that have shown any promise in predicting potential for environmental harm". Biological assays can provide evidence as to whether or not waste materials added to the environment may be toxic regardless of whether or not their chemical composition is known.

The types of bioassay tests used for assessing the toxicity of industrial discharges, with reference to solid wastes and bioassays carried out in the Arctic are reviewed below.

2. LITERATURE ON BIOASSAY METHODS

There is a large number of reviews on the methodology of toxicity testing. Waldichuck (1973) shows how biological asssays fit into the various procedures for assessing the impact of pollutants on an ecosystem and Pessah and Cornwall (1980) discuss the use of toxicity testing of industrial effluents in the context of meeting the requirements of Canadian regulations. Sprague (1973) and Davis (1975) give good overall introductions on the subject of what variables should be considered before embarking on bioassays and Standard Methods (APHA, 1980) gives detailed accounts on conducting bioassays to meet regulatory requirements in the United States. A brief but easily comprehensible account of methodologies can also be found in a work published by the U.S. Environmental Protection Agency (Stephan, 1975). Alternative procedures, some of which are still in the research and development stage, are described in Scherer (1979). Although these new biological assays use fresh water organisms, the concepts could also be applied to studies of organisms in the marine ecosystem. Descriptions of bioassay procedures for marine organisms can be found in Cox (1974). Gehrs et al. (1979) discuss how bioassays combined with chemical procedures can be used to determine the lethal ingredient in a complex mixture. Perkins (1972) recounts some of the steps that should be taken to ensure that field collected specimens are suitably healthy for bioassay studies. Reish (1973) suggests that since the problems discussed by Perkins are somewhat difficult to resolve it is more advantageous to use laboratory-raised populations which then can become "international standards". Descriptions for culturing invertebrates can be found in Galtsoff et al. (1959). Fisheries and Oceans Canada publishes the proceedings of Aquatic Toxicity Workshops held annually in Canada (e.g., Kaushik and Solomon, 1983). Reviews of the most recent literature on biological assays are presented in a yearly issue of the Journal of the Water Pollution Control Federation (e.g., Little, 1976; Maciorowski et al., 1981, 1982, 1983). Craddock (1977) has compiled data on the results of bioassays designed to test the toxic effects of petroleum on arctic and subarctic species.

3. BIOASSAY TERMINOLOGY

Descriptions of various terms associated with conducting bioassays can be found in Sprague (1973) and Standard Methods (APHA, AWWA, WPCF,1980). Some of the more common terms are described in the following sections.

3.1 Toxicity Terms

An **acute toxicity test** can be defined as a test in which a toxicant has a sufficiently high concentration to bring about a response (lethal or otherwise) or effect within a short period of time (normally 96 hours) in fish and macroinvertebrates. An acute toxicity test is a short-term test. The goals of an acute toxicity test can be either to determine the relative toxicity of a substance, discover if it is toxic to a particular species, or to help the investigator set up the appropriate conditions for long-term tests. It is the most common procedure for determining the toxicity of substances to small populations over a short length of time. It forms the "backbone" of the procedures used for regulatory purposes (Monk, 1983). An acute toxicity test of an unknown substance represents an initial screening test done under numerous experimental conditions with a variety of organisms to obtain an early estimate of its relative toxicity (Maki and Duthie, 1978). The concensus is that acute toxicity tests are considered to be ecologically significant, legally defensible and cost effective (Buikema et al., 1982).

A **sub-acute toxicity test** is a test in which the concentration of the toxicant is less than that used in an acute test so that a longer time is required to produce a response or effect (Sprague, 1973). Subacute toxicity tests may be partial life cycle tests of an organism and thereby help to determine if a particular life stage is more sensitive to a toxin than another.

A **chronic toxicity test** is a test in which the concentration of the toxicant is so low that the test organisms are able to survive a long time (possibly one-tenth of their normal life span) so that long-term effects such as changes in growth, metabolism and reproduction in addition to mortality can be observed. Sprague (1976) concluded that the most sensitive and productive experiments were those studying the chronic effects of pollutants on the reproductive cycles of organisms.

The term **dose** has been defined in Standard Methods (APHA, AWWA, WPCF, 1980) as the "amount of toxicant that enters the organism". When working with invertebrates in which it is difficult to measure the internal concentration of the test product the term "dosage" can be considered as the product of the concentration of the substance and the time it is administered to the test organism. Acute doses of substances are concentrations within the organism causing death over a short period of time, in contrast to a chronic dose which acts over a long period of time at a low concentration. It should be emphasized that the response of an organism (the mechanism leading to death) to a chronic dose might not be the same as that to an acute dose. Dosage is rarely determined in bioassay studies using aquatic organisms because the concentration within the organism is not determined. Instead concentrations in the medium are measured.

The term **LC50** refers to the lethal concentration of a substance killing 50% of the exposed organisms over a specific time of observation. Hence the symbol must be accompanied by a specified time period. For acute tests the time period is usually 96 hours. Hence an acute concentration would be 96h-LC50 or the concentration of a substance killing 50% of the organisms by 96 hours.

The term **EC50** refers to the effective concentration of a substance at which a specific response such as a behavioural or physiological change other than mortality is manifested over a specified time period, e.g., 96h-EC50. For instance, the ninety-six hour effective concentration might be a useful datum to know for a chronic toxicity test.

3.2 Flow Terms

The flow terms are used to describe the movement of water and whether or not the toxicant concentration is replenished to a constant level during the bioassay. A rationale for deciding which system to use is shown in Figure F.1.

a) Static Bioassay

The test organisms are exposed to the same test and control solutions for the duration of the test. This is the simplest and least costly of the bioassay procedures. Of the available tests, static bioassays often bear the least resemblance to the conditions found in nature, but in some instances they may approximate phenomena such as a once only dump or spill of material. This system is often used when preliminary or screening data are required.

b) Recirculation Bioassay

This is a static bioassay in which the test solution is circulated through the test chamber. The circulated water may be filtered, aerated or stirred. This system is often used when a constant supply of water is not available and the bioassay is expected to run for a time longer than 96 h.

c) Static Replacement Bioassay (Renewal Bioassay)

This is a static test except that the test and control solutions are periodically replaced by solutions of the same composition. This is simpler than a recirculation system but used for the same reasons.

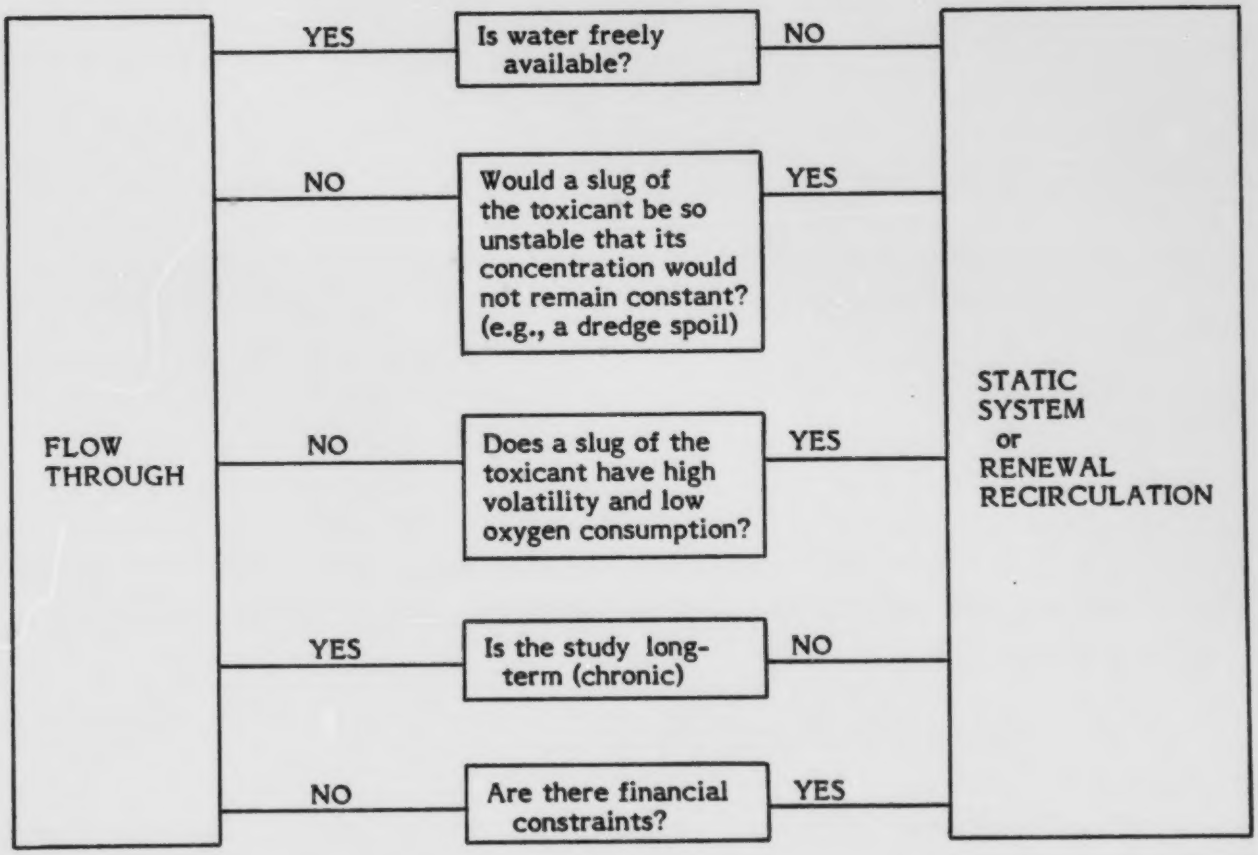
FLOW THROUGH
YES
Is water freely available?
NO
NO
Would a slug of the toxicant be so unstable that its concentration would not remain constant? (e.g., a dredge spoil)
YES
NO
Does a slug of the toxicant have high volatility and low oxygen consumption?
YES
YES
Is the study long-term (chronic)
NO
NO
Are there financial constraints?
YES
STATIC SYSTEM or RENEWAL RECIRCULATION

Figure F.1 General Principles and Concepts of Selection of Flow System

d) Flow-Through Bioassay

This is a test in which fresh test solution flows continually through the test chamber with the intention of continually exposing test organisms to a constant concentration of the toxicant. This type of bioassay is the most complex of the procedures and the most costly. The rationale for this system is that it is considered to provide the most accurate data for long-term studies.

e) *In-situ* Bioassay

Occasionally toxicity experiments are carried out *in-situ* using organisms exposed to the environment in which they can be expected to live. The expectation is that the artificial restraints of the laboratory are removed and the organism provides an integrated response to all environmental variables. The data can be difficult to interpret because of the large number of variables.

Procedures for the recirculation and flow-through bioassay systems are discussed in detail in the proceedings of a workshop on marine bioassays (La Roche, 1974).

4. USES AND LIMITATIONS OF BIOASSAYS

As mentioned earlier, a main objective of a bioassay can be to predict the effect of disposal or dumping of a substance or substances on living organisms in the marine benthic environment. Since this ideal is difficult to achieve, bioassays usually concentrate on more limited goals. These apply under laboratory conditions only, and include:

a) determination of the concentration of a substance necessary to kill some percentage (almost always 50%) of a test organism over a specified interval of time (usually 48 h or 96 h for acute toxicity tests);

b) determination of the relative sensitivities of various species, especially those native to a discharge area or possibly of commercial value, to the same toxicant under laboratory conditions;

c) determination of the relative toxicity of two or more toxicants to the same species;

d) determination of how the potency of the toxicant alters with differing environmental conditions;

e) determination of what fraction of the toxicant has the most toxic properties;

f) determination of the effect of environmental factors on waste toxicity;

g) determination of permissible effluent discharge rates;

h) initial estimate of the toxicity of a toxicant to different life stages of a species.

The main limitations in interpreting the results of a bioassay, especially an acute one, are (Anon, 1979; Brungs and Mount, 1978):

a) the data may not be indicative of the sensitivity of all the organisms living at the site of environmental impact or waste disposal;

b) the data may not be indicative of a species' sensitivity at different stages of its life cycle;

c) the 50% criterion for measuring potency of the test material in a 96h-LC50 test is unacceptable from an ecological standpoint since 50% of the species could be expected to die;

d) the sublethal effects of exposure may linger long after the experiment has stopped, thereby incorrectly estimating the actual toxic concentration of a substance.

Despite these shortcomings, the results of bioassays can warn of a potential problem, suggest further study and indicate caution in the use or disposal of the test substance.

5. PRACTICAL ASPECTS OF BIOASSAYS

The type of bioassay to be conducted depends upon the goal of the bioassay, the materials available to the investigator and the composition of the toxicant. For example, a bioassay with the modest goal of testing the relative sensitivities of various species to a single toxicant with conditions remaining constant from one experiment to the next will not require the same degree of replication of environmental conditions as one with a more ambitious goal (such as determining permissible discharge rates). Generalizing the results from the laboratory to the environment are mainly dependent on how closely the assay models the conditions in the environment. A chronic bioassay test would be appropriate, for example, if the investigator wishes to predict the possible effects of material being discharged at low concentrations over a long period of time while an acute toxicity test would be appropriate if the effluent is discharged as a slug (Brungs and Mount, 1978). Acute toxicity tests would be inappropriate for investigating the potency of a mutagen which requires a long time to act before its effects are manifested in the exposed organisms.

Table F.1 shows that four basic types of bioassay studies can be conducted depending upon whether or not a flow-through system is required and whether or not a long-term or short-term test will provide the appropriate data. The decision on which combination of flow system and time for doing the experiment is also determined by the type of water supply the investigator has available for running the assays. For example, if pollutant-free water is freely available and if a continual supply can be made available to the test organisms then FLOW-THROUGH assays can be undertaken, but if the water supply is restricted, RENEWAL or STATIC systems are the only choices. If a FLOW-THROUGH system is available then the investigator can decide whether or not to carry out acute assays or long-term assays. The type of assay conducted also depends upon the type of toxicant. If the toxicant is stable over a relatively long period of time then LONG- or SHORT-TERM studies can be undertaken. Otherwise studies will be restricted to either INTERMEDIATE or SHORT-TERM assays unless a FLOW-THROUGH system is available. The advantages

TABLE F.1

THE MAIN COMBINATIONS OF TIME TO OBSERVE RESULTS AND TYPE OF WATER SUPPLY FOR RUNNING BIOASSAYS

	ANALYSIS TIME	
Water Supply	**Short**	**Long**
	Acute Test	**Chronic Test**
Static Test water is not changed	(simple, rapid, high utility, well documented)	(short-lived invertebrates)
	Acute Test	**Chronic Test**
Renewal or flow-through Test water is changed	(Screening unstable materials, better estimates of toxicity)	Observation of: (1) long-term mortality (2) sublethal (behavioural, physiological, and biochemical) effects.

of a FLOW-THROUGH system is that the experiment can be conducted under more constant conditions, the test organisms can be exposed for longer periods of time since they can be fed and the effects of the toxicant on different stages of the life cycle can be evaluated (Brungs and Mount, 1978). The advantage of a static system is that it is much simpler to run and maintain and hence less expensive. Sprague (1973) noted that flow-through tests are not necessarily superior to a well-done static test if there are problems with the test substance settling out, coagulating or otherwise providing an accurate and constant concentration. For example, a sealed static assay chamber with no head space and a sufficiently low loading density is best for studying the toxicity of the volatile oil hydrocarbons provided that the oxygen is not drastically consumed. A static test can provide valid results at costs lower than those obtained by using a flow-through system.

The terms short, intermediate and long are relative and are dependent upon the life span of the test organism. Standard methods (APHA, AWWA, WPCF, 1980) states that short-term tests range between 24 and 96 hours with 96 hours being recommended for fish and 48 hours being recommended for the water flea, Daphnia sp.. The Environmental Protection Service recommends a 72 to 96 hour exposure for acute lethality using Daphnia. The advantage of the 96 hour limit is that the toxicity of many substances does not substantially increase after this time (Monk, 1983). Intermediate tests (considered to be 8 to 90 days long) and long-term tests (possibly lasting over the life span of the test organism) determine the effect of a toxicant over the life cycle or partial life cycle of an organism.

Acute toxicity tests range in cost from about $ 100 to $ 300 per test while chronic tests, depending upon their level of sophistication can range from $ 500 to $ 500,000 per test (Branson et al., 1981).

A flow chart indicating some guidelines for setting up a bioassay is shown in Figure F.2. Figure F.1 is a flow chart depicting the selection of an appropriate flow system. Examples of studies using various experimental designs are given below.

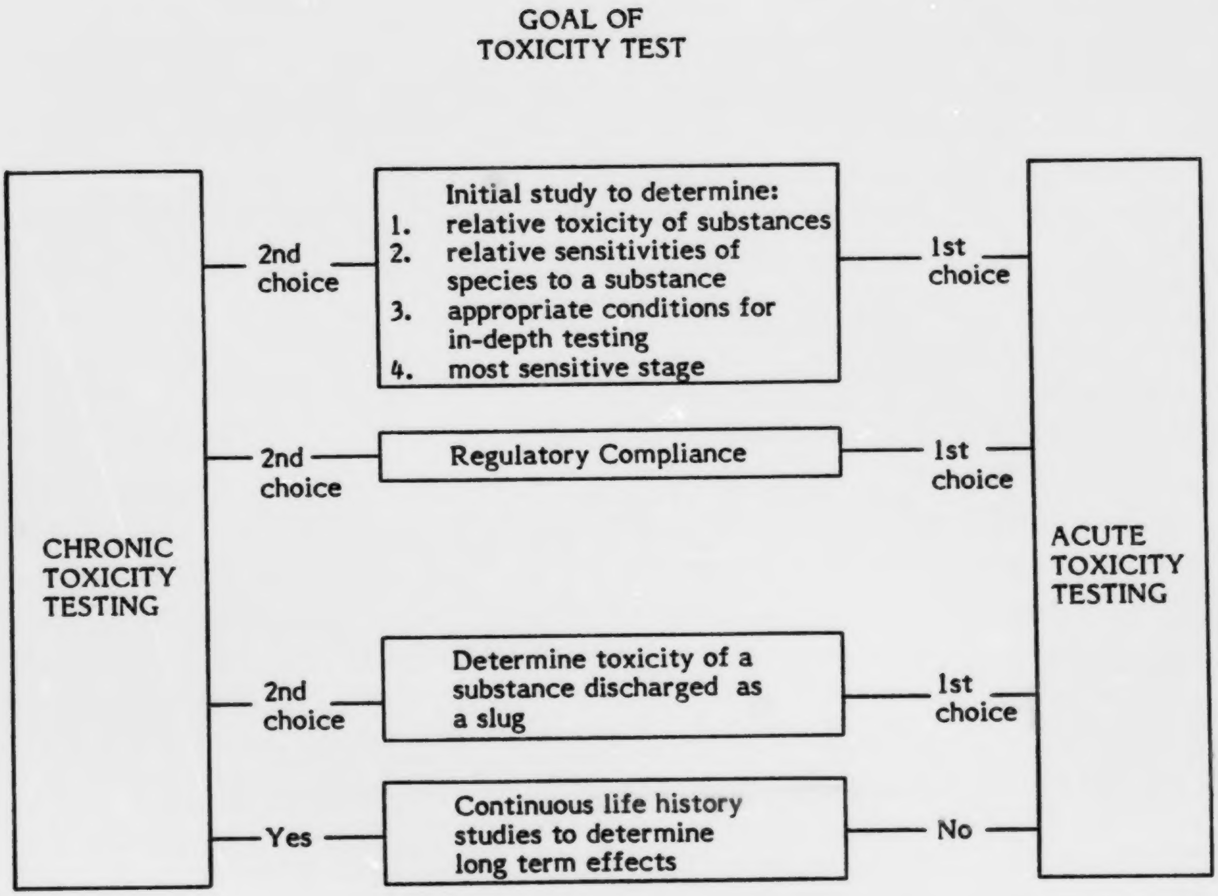
GOAL OF
TOXICITY TEST
CHRONIC
TOXICITY
TESTING
2nd choice
Initial study to determine:
1. relative toxicity of substances
2. relative sensitivities of species to a substance
3. appropriate conditions for in-depth testing
4. most sensitive stage
1st choice
2nd choice
Regulatory Compliance
1st choice
ACUTE
TOXICITY
TESTING
2nd choice
Determine toxicity of a substance discharged as a slug
1st choice
Yes
Continuous life history studies to determine long term effects
No

Figure F.2 Toxicity Test Selection

5.1 Experimental Designs for Bioassays

5.1.1 Acute Toxicity/Static System

A recent study in the Alaskan Beaufort Sea carried out acute toxicity tests in a static system to determine the relative toxicities of differing compositions of drilling fluids to various species of arctic organisms as part of a program to study the environmental impact of offshore drilling fluid disposal (Tornbers *et al.*, 1980; NORTEC, 1981). In this study acute toxicity assays (e.g., 96h-LC50 tests) were conducted by exposing test organisms to unreplenished water containing varying concentrations of drilling fluids and measuring mortality. An acute toxicity experiment using the amphipod, *Onisimus* sp., was carried out by setting up six test tanks of 1L capacity each with ten specimens per tank and varying concentrations of the drilling fluid, XC-Polymer/Unical (0%, 15%, 25%, 30%, 35%, 45%). The mortalities at 0.5 h, 4 h, 8 h and so on up to 96 hours were observed. These data were then used to calculate the concentration of drilling fluid expected to kill 50% of the test organisms over a 96 hour period. Another 145 similar assays were carried out. The report lists the salinity, oxygen concentration, temperature of the water for each bioassay and includes data for "reference" materials, which in this case were barite and lignosulfonate. As a result the investigators were able to rank the relative toxicities of the various drilling fluids as well as the sensitivity of various species to similar drilling fluids taking into account the other variables in their experiments. The results suggest that mysids (presumably *Mysis relicata*) have approximately the same sensitivity to drilling fluids as do fish with respect to acute lethality tests. The study concluded that acute toxicity effects were not expected to occur in the Arctic.

5.1.2 Acute Toxicity/Flow-Through System

Pulp mill effluents are common pollutants containing chemical mixtures, some of which are volatile. In a two-year study Davis and Mason (1973) determined the acute (96 h) toxicity of bleached kraft mill effluent to three species of juvenile salmon (kept at various population densities) using both a static and flow-through system. The results of this study showed that the effluents were less toxic in static systems than in flow-through systems presumably because volatile substances were being eliminated in the static system. In addition, the results showed that fish density was an important factor in estimating toxicity. The investigators were able to show that storage of the sample also decreased its toxicity.

5.1.3 Chronic Toxicity/Static System

A chronic toxicity testing procedure using a static system has been described by Busdosh and Atlas (1977). These investigators studied the long term (chronic) toxic effects of exposure to different types of oil or to different fractions of oil to two species of arctic amphipods, Gammarus zaddachi and Boeckosimus (Onisimus) affinis, by determining the time it took for 50% and 100% of the test organisms to die as a result of exposure to a single concentration of toxicant. The experiments were conducted at 5^{o} C. The testing system was either a static system using 45 cm by 20 cm trays of 5L capacity holding thirty amphipods per tray or in situ, (the test organisms were enclosed in cases, 50 per case, from which they could not escape and suspended in an experimental pond). The use of open trays was justified in this experiment since evaporation is reduced at low temperatures. The authors concluded that spillage of crude and refined oils in the Arctic would cause large scale mortality of Arctic amphipods.

5.1.4 Chronic Toxicity/Flow-Through System

Ward et al. (1979) describe a bioassay method using the marine copepod, Acartia tonsa, which has a thirty-day life span and can be cultured under laboratory conditions. In their study 100 nauplii larvae were placed in 1L of test media (three concentrations of a chemical waste plus a control) held in dishes of 2L capacity. The test media were replaced using a metered flow-through system at a rate of 1000 mL every twelve hours. The test organisms were fed every two days with algae. The number of test organisms surviving the test solutions was counted at the end of twenty days. The results of this study indicated that Acartia tonsa is a good organism for chronic toxicity testing of materials deposited into marine environments because of its short generation time, small size, sensitivity and prolific nature.

5.2 Consideration of Variables Associated with Quality of Bioassay Water

The characteristics of the dilution water need to be considered when performing toxicity tests. Variables such as salinity, pH, turbidity, temperature and dissolved oxygen ideally should be measured and reported for every experiment. In the Arctic, near bottom water in all regions will generally have temperatures in the range of -1.8° C to 0° C and a salinity of 30 to 34 °/oo. Hence if natural ambient conditions are to be used to simulate arctic conditions then water temperature needs to be kept quite low. It has been strongly emphasized by Sprague (1973) that there must not be any artificial oxygenation of the water until it is proven that such a procedure does not degrade or remove the toxicant. The pH of the medium must be monitored if the potency of the toxicant is pH-dependent as is the case with ammonia and cyanide. The water temperature should be maintained within a range of $\pm 1.0^{\circ}$C of the desired temperature. If the purpose of the test is to determine whether or not a particular substance is toxic as compared to another substance then the concern about water temperature may not be necessary, but if the purpose of the test is to help predict the impact of the substance on organisms in a particular ecosystem then the influence of the ambient temperature does become important. Since the metabolic rate of an organism increases with temperature, and provided that the organism is acclimated to a particular experimental temperature within its zone of tolerance, it may be more practical to run experiments at warmer temperatures than at colder ones with the proviso that temperature is not acting as a stressor. Similarly the salinity of the water must be considered. For example, Perkins (1972) reports that the starfish Asterias rubens will survive indefinitely but will not grow at a salinity of 20 °/oo but will grow at a salinity of 30 °/oo. Finally, the effect of turbidity should be considered as some toxicants are absorbed onto particles. If these particles are not ingested by the organism than the concentration of the chemical has been effectively reduced (is less biologically available) with the consequence that its toxicity would be underestimated.

6. QUALITY ASSURANCE

An evaluation of the quality of bioassay data is required in order to be able to compare with confidence data from various laboratories. A problem with bioassay tests is that the evaluation of the quality of data is hampered by the difficulty of being able to validate and standardize tests. With the possible exception of laboratory-bred mammals there are no standard reference marine species analogous to reference materials available for chemical analyses and no measures of accuracy. The quality of bioassay data must therefore be assessed by more general means. The validity of a bioassay is enhanced if a material is tested under a range of conditions, with a variety of species and possibly a reference toxicant. However, the cost increases substantially as the number of experimental conditions, species and reference toxicants are increased. If the bioassay is used for the purposes of screening or routine monitoring, these additional costs may prove prohibitive.

Branson et al. (1981) have suggested that despite using the same conditions, toxicant and test organism, within lab differences could be an order of magnitude while among lab differences would be greater. For example, Buikema et al. (1982) report that interlaboratory estimates of the LC50s of an effluent using a "standardised" procedure varied among the three labs from 2.5 to 38.5% effluent. One reason for the lack of agreement is that there are a large number of variables which enter into each study of which only a portion are actually controlled or remain constant. For example, it is difficult to ensure that the test organisms used in a bioassay among labs have the same genetic composition and are in the same state of acclimatization (Vernberg and Vernberg, 1972). In addition, it is unlikely that the dilution water for the bioassays has an identical composition among labs.

A workshop (Bowman, 1982) on evaluating documentation quality recommended a number of useful criteria to aid in making a judgement and the AQUIRE (AQUatic Information REtrieval) data base have listed a comprehensive compilation of toxicity criteria. The following summary has been taken from these sources. Different "quality indicators", relating to methodology, data evaluation, source (such as original work), and level of accuracy reported for the chemical or physical data can be considered in evaluating the quality of bioassay data and papers can be assigned a numerical rank depending upon how well the criteria are met. Bioassay data in computer-stored data bases could be "'tagged" by labels for each of these quality indicators.

Some criteria for **"methodology"** quality indicators are:

1) that the test organisms:
   - originate from the same source
   - are the same hybrid

- are approximately the same age and size
- are cultured at approximately the test conditions
- are acclimatized to test conditions (salinity, temperature, photoperiod)
- are disease free
- have a regular feeding schedule of known food type and quantity.

2) that the design of the experiment:

- includes controls
- exposure details (method of administration, solvent, duration/ sequence)
- ensures that control mortality does not exceed ten percent
- ensures that a control for any solvent be included
- uses the appropriate type of dilution water
- includes details on the test system (vessel description, volume, depth)
- states exposure duration and dates
- states the number of test organisms per vessel
- states the concentrations established.

3) the quality of the test material should be evaluated in terms of:

- sampling details
- sample type (grab, composite)
- sample source
- sample description
- collection date
- storage/shipping details
- date/time received in laboratory.

With respect to **"data evaluation"** quality indicators, the information needed is what type of review the data was subjected to (critically evaluated, journal/peer review, internal review, no review) before being published (Bowman, 1982).

Some measure of **accuracy** for chemical and physical data must be reported. Buikema et al. (1982) are particularly critical of assays in which the test concentrations of the toxicant are not verified and consider them to be of limited use in monitoring discharges. Quality of chemical analyses has been discussed previously in Section III-1. The material being assayed is usually a complex mixture of many substances so that chemical determination of the concentrations of each of the substances is generally not practical. Although the evaluation of bioassay data quality is a subjective exercise, confidence in this evaluation and hence the data would be enhanced if the format of testing followed that given in the publications by the University of Wisconsin (CLSES, 1984; Figure F.4).

7. DATA ANALYSIS

7.1 Experimental Design

This section is based on the assumption that the investigator is interested in reporting data on a dichotomous variable such as mortality (e.g., toxicity tests in which LC50's are calculated; organisms are either dead or alive), rather than data on a continuous effect.

Detailed discussions of the statistical treatment of the results of toxicity tests can be found in texts by Ashton (1972) and Finney (1978) and reviews (Sprague 1969, 1973; Stephan, 1977; Buikema *et al.*, 1982). Sprague (1973) emphasizes graphical methods while the others emphasize mathematical treatments of the data. The advantage of a graphical method is that an LC50 value is easy to obtain provided that an estimation of its variance is not needed. Stephan (1977) also points out that a single experimental determination of an LC50 value does not provide any indication of the precision of the test and this can be obtained only by repeating the experiment. He also argues against the need for conducting tests in which it is necessary to obtain partial kills since the resulting data can be analysed using non parametric methods. Parametric methods are based on assumptions about the theoretical distribution of the data and hence require results in which not all of the organisms survive or die. Stora (1974) demonstrates a useful statistical procedure for using specimens which, as a result of being collected from the field, are probably not genetically homogeneous. This procedure would be useful for a long term study in which the same species could be expected to be used often and it is important to determine if the resulting LC50 values differ as a result of different toxicities of the test material rather than differing sensitivities of the test organisms. The text by Finney (1978) is especially useful in that it discusses the merits of using the various statistical procedures. Buikema et al. (1982) provide a detailed discussion on data interpretation.

All experiments are constrained by the availability of a number of resources such as time, money or experimental subjects. The object of an experimental design is to optimize the chance of obtaining accurate and precise data often with limited resources and without knowing anything about the material to be investigated. Such optimization could be approached by undertaking the following procedures.

1) Randomly assign the experimental organisms to the treatments. An easy way for bioassays would be to number each vessel, pick one specimen and then assign it to a vessel selected using a random numbers table.

2) Run controls simultaneously with the experimental treatments. Control mortality must remain below 10% if the assay is to be of value. Higher values suggest that some stress factor may be present which would enhance the sensitivity of the experimental organisms to the toxicant.

3) Use a few test organisms per experimental treatment and have many treatments instead of having a few treatments with many test organisms per treatment. The advantage is that data on zero kills, partial kills and complete kills may be obtained with a resultant greater choice of statistical treatments.

4) Use equal numbers of experimental organisms for each treatment. The results of the assays if this is done are more easily analysed. Many of the formulae used in the statistical analyses are simplifed.

5) Have the concentration of test substances progress in a geometric (logarithmic) series rather than in an arithmetic one as this optimizes the range of statistical analyses available (some require that the doses be evenly spaced in a geometric series).

6) If there is some notion of the LC50 then plan the doses symmetrically around it as there is an increase in the precision of the calculated results.

7) Use reference toxicants (eg. Cardwell et al, 1977; Davis and Hoos, 1974) as method of establishing that the results are due to the toxicant alone rather than due to a combination of the toxicant and the test conditions.

8) Try to "standardise" bioassays in order that the replication among labs can be easily accomplished. Such replication may lead to improved accuracy in data analyses.

7.2 Treatment of Data

There are a number of statistical methods which can be applied. The most practical for bioassays involving toxicity testing of new materials may be the following non parametric methods:

1) the Spearman-Karber method (Irwin and Cheeseman, 1939; Cornfield and Mantel, 1950),

2) the moving average method (Thompson, 1947), and

3) the binomial method (Stephan, 1977).

These methods do not require data on at least two partial kills. Each of these methods has a weakness; the Spearman-Karber method requires that data on 100% and 0% kills be available, the moving average method requires that there be at least one partial kill and that the doses be geometrically spaced, and the binomial method does not actually yield a unique value of the LC50. If data on at least two partial kills are available then such techniques such as probit and logit analyses are applicable, provided that a computer is available. An easily followed technique which is partly graphical and requires data on at least two partial kills (preferaby between 40 and 60%) is the Litchfield-Wilcoxon (1949) method.

While both Stephan (1977) and Finney (1978) have discussed the merits of the various techniques they did not actually show the mechanics of determining the values for the LC50 data and the 95% confidence intervals (C.I.). Descriptions of determining the LC50 and 95% C.I. using the Moving Average Method, Spearman-Karber method and the Litchfield-Wilcoxin method are presented below:

Numerical examples for calculating LC50's using different procedures:

The data set is test number 24 on Table 4 of Stephan, 1977. There are ten organisms per treatment.

i	Dose	Log Dose	% Mortality	Probability p
	Control		0	0
1	7.776	0.891	0	0
2	12.96	1.112	0	0
3	21.60	1.334	20	0.2
4	36.00	1.556	70	0.7
5	60.00	1.778	100	1.0
6	100.00	2.000	100	1.0

A. Moving Average Method:

References:

Thompson (1947), Finney (1978)

Definitions:

k: the span of the moving average (number of data points)

d: the difference between successive doses (log transformed)

m: the value of X at $p = 0.5$

Conditions:

1) equal spacing of doses
2) $p_i \neq p_{i+k+1}$
3) p-trial function $\left(p_i + \dots + p_{i+k+1} - \frac{nk}{2}\right)$ lies in the interval (p_i, p_{i+k})

For the purposes of this demonstration $k = 3$ (as it has been found to yield high precison).

$$p' = \frac{(p_i + \dots + p_{i+k-1})}{k} \qquad p'' = \frac{(p_{i+1} + \dots + p_{i+k})}{k}$$

$$x = \frac{(x_i + \dots + x_{i+k-1})}{k}$$

if $p' \neq p''$, $p' \leq 0.5 \leq p''$ and treatment sizes are not equal then m can be estimated by

$$m = x' + d \frac{(k-1)}{2} + d.f.;$$

where $f = \left(\frac{k}{2} - p_i - \dots p_{i+k-1}\right) / (p_{i+k} - p_i)^2$

or by

$$m = x' + d.f.;\ \text{where } f = (0.5 - p') / p'' - p')$$

when treatment sizes are equal.

Given that n = 10 is constant and i = 2 then

$$p' = \frac{0 + 0.2 + 0.7}{3} = 0.3$$

and

$$p'' = \frac{0.2 + 0.7 + 1.0}{3} = 0.63$$

which surrounds p = 0.5.

The value of the dose corresponding to p' is

$$x' = \frac{1.112 + 1.334 + 1.556}{3} = 1.482$$

The LD50 is estimated as follows:

$$m = 1.482 + 0.2218 \times \frac{0.5 - 0.3}{0.63 - 0.3}$$

$$= 1.468$$

antilog m = 29.35

The variance ($v_{\log m}$) about m is estimated by:

$$V_{\log m} \simeq d^2 \cdot \sigma_f^2$$

where

$$\sigma_f^2 = \frac{1}{(\bar{p}_{i+k} - p_i)^2} \times \left[(1-\bar{f})^2 \cdot \sigma^2_{p_i} + \sigma^2_{p_{i+1}} + \cdots \sigma^2_{p_{i+k-1}} + \bar{f}^2 \cdot \sigma^2_{p_{i+k}}\right]$$

where

$$\sigma_i^2 = v_i = \frac{p_i \cdot q_i}{n - 1}$$

$$\simeq 0.2218^2 \times \frac{1}{(1-0)^2} \times \left[(1-0.6)^2 \cdot \frac{0.1}{9} + \frac{0.2 \cdot 0.8}{9} + \frac{0.21}{9} + 0.6^2 \cdot \frac{10}{9}\right]$$

$$\simeq 0.002$$

The 95% C.I.

$= m \pm 2\, v^{1/2}$

$= 1.468 \pm 2\,(0.447)$

$= .378$ to 1.557

Antilog values are 23.9 to 36.1

B. Spearman - Karber Method

References

Irwin and Cheeseman (1939); Cornfield and Mantel (1950); Finney (1978).

Restrictions:

That there be a 0 and a 100% kill in the treatments.

Listed below are the generalized formulae for unequal n's for the treatments.

$$m = \sum_{i=z}^{s} (p_i - p_{i-1}) ((x_i + x_{i-1})/2); \; i=2 \text{ to } s$$

$$= (0 - 0) \times ((1.112 + 0.891)/2) +$$

$$(0.2 - 0) \times ((1.334 + 1.112)/2) +$$

$$(1 - 1) \times ((2 + 1.778)/2)$$

$$= 1.468$$

Antilog m = 29.35

The variance of m (σ_m^2) is

$$\sigma_m{}^2 = \sum_{i=z}^{s} (x_{i+1}) - x_{i-1})^2 \times ((p_i)(q_i)/(n-1))$$

$$= (0.222)^2 \times ((0)(1)/9) +$$

$$(0.222)^2 \times ((0.2)(0.8)/9) +$$

$$(0.222)^2 \times ((0.7)(0.3)/9) +$$

$$(0.222)^2 \times ((1)(0)/9)$$

$$= 0.0020$$

Therefore the 95% C.I. is 23.9 to 36.1

C. Litchfield - Wilcoxon Method

Reference

Litchfield and Wilcoxon (1949)

Restrictions:

Requires at least two partial kills.

This is a graphical technique requiring probability paper and a table of values known as corrected probits. The method is clearly described in the reference. The results of the method are shown in Figure F.3.

Methodology:

1) Plot on probit-log graph paper omitting p's less than 0.05 and greater than 99.95

2) Draw "free hand" a line equally close to all points particularly those between 40% and 60%.

3) In this example effective use of 100% mortality data can not be made because the line is too steep.

4) LC50 is extended to be 32

5) To calculate 95% C.I.
   a) determine slope function, S and a factor (denoted $f_{LC_{50}}$)
   b) read from regression line the expected LC_{16} and LC_{84} if these are approximately 0.21 and 39 respectively.
   c) determine the number of individuals used between 16 and 84% and denote N

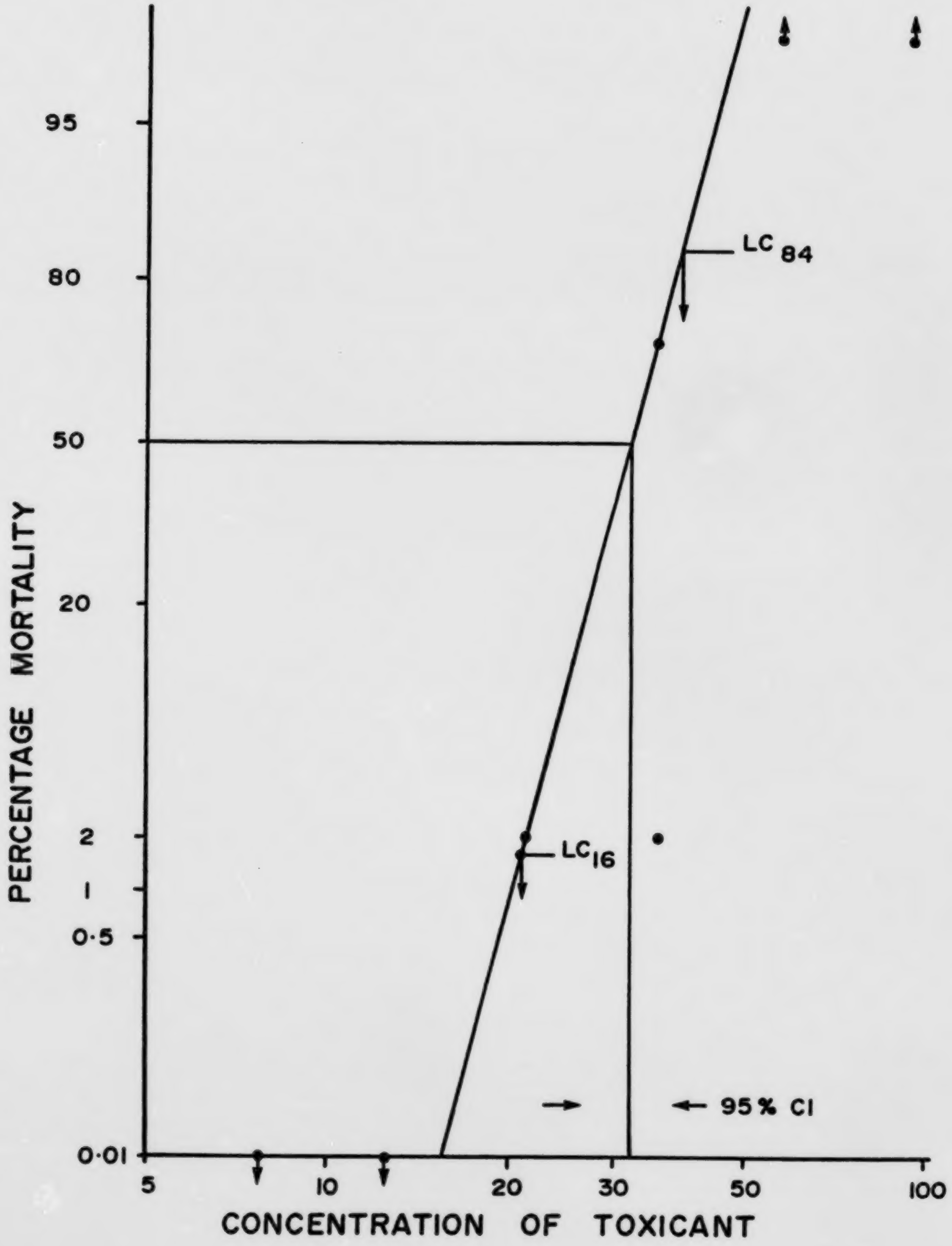

Figure F.3 Litchfield and Wilcoxon (1949) graphical estimation of LC50.

d) $$s = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$$

$$= \frac{39/32 + 32/21}{2} = 1.37$$

e) $f LC_{50} = S.^{2.77/\sqrt{N}} = 1.37^{2.77/\sqrt{20}}$

f) upper and lower 95% C.I. are equal to:

$LC_{50}/f LC_{50}$ and $LC_{50} \times f LC_{50}$ respectively

= 26.3 to 38.91.

A summary of the resulting analyses are given below in Table F.2.

Table F.2

Summary of Resulting Analyses of Acute Toxicity Data

Method	96 hr LC50	UCL	LCL
A. Moving Average	29.35	36.1	23.9
B. Spearman-Karber	29.35	36.1	23.9
C. Litchfield & Wilcoxin	32	38.91	26.3

8. APPLICATIONS OF BIOASSAYS TO SOLID WASTES

The procedures described above were developed primarily for testing the toxicity of liquid effluents. Test procedures for determining the acute toxicity of solids such as mine tailings and dredged materials have required some modification of the basic methods. It is possible to test three components of marine sediment:

1) a liquid phase in which an elutriate of the solid is tested for toxicity,

2) a suspended phase in which the fine solids suspended in water are tested, and

3) a solid phase in which the toxicity of the most dense portion of the sediment is tested (Alden and Young 1982).

Static, replacement and flow-through assays have been undertaken to determine the effects of solids in the three phases (Swartz et al., 1979, Alden and Young, 1982, Swartz et al., 1982). Flow-through systems described by Swartz et al. (1979) and Rubenstein et al. (1980) seem to be well suited for testing the toxicity effects of various sediment phases, but are also elaborate. Prater and Hoke (1980) describe an interesting recycling system which requires little capital expenditure but can be used for testing the toxicity of sediments on invertebrates and fish. Toxicity was expressed as percent mortality after ninety-six hours. Pedicord (1980) describes a system for determining the toxicity of suspended solids using a recirculating seawater system. In this system a contolled and constant amount of slurry could be introduced into each aquarium holding the test species.

8.1 Requirements for Regulatory Compliance Testing

There are at present no guidelines specifying a bioassay procedure for testing the toxicity of sediments or solid wastes in arctic regions. Guidelines for testing the toxicity of liquid effluents discharged into freshwater and seawater are documented by the Environmental Protection Service (EPS, 1980) and by the British Columbia Ministry of the Environment (1982). Pessah and Cornwall (1980) have described the background and intent of the EPS document. The bioassay procedure is based on an acute (96 h) toxicity test using healthy and acclimated stocks of the trout, Salmo gairdneri, ranging in weight between 0.5 g and 10 g, and numbering at least five per test solution, its volume at least .5 liter per gram of fish for every twenty-four hours of testing. Guidelines for controlling or measuring various environmental variables are also given. The EPS document explicitly states that for determining the effects of freshwater discharges into seawater the test organism is to be the rainbow trout, Salmo gairdneri, but if the discharge is saltwater (salinity greater than 10 ppt) then the test organism is to be a fish species authorised by the Minister of the Environment. For the purposes of regulatory compliance or prosecution it is necessary to demonstrate that the test species could conceivably be found in the waters in question (Rankin and Leaman, 1983).

Data used for regulatory compliance or in litigation are required to show a chain of continuity of possession of the samples being assayed (Section II-9). Another consideration is that the law courts prefer results which consist of mutually exclusive categories (e.g., alive versus dead) as they are easily interpreted (Rankin and Leaman, 1983).

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Volume

2

SAMPLING AND ANALYSIS
IN THE ARCTIC
MARINE BENTHIC ENVIRONMENT

VOLUME 2
GUIDE TO PRACTICE

Prepared for:
Environmental Protection Service
Yellowknife, N.W.T.

by:
Arctic Laboratories Ltd.
March, 1985

SAMPLING AND ANALYSIS IN THE ARCTIC MARINE BENTHIC ENVIRONMENT

VOLUME 2

GUIDE TO PRACTICE

The Environmental Protection Service wishes to encourage user comments and suggestions on the content and utility of this guide. Any comments concerning the content of this report should be directed to:

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Additional copies of this report and the companion report (Volume 1; Review of Methods) can be obtained from the above address at an estimated cost of $25.00 per report.

PREFACE

This is the second part of a study to produce a guide for managers and planners involved in the collection and analysis of environmental data from marine sediments in arctic regions. The first phase of this study (Volume 1) reviewed existing methods and was intended to serve as a background document to the present report.

This report is intended to provide guidance in planning and in choosing appropriate methods for a variety of situations, conditions and objectives. The format is designed to outline in a step by step manner the factors to be considered and offers suggestions as to possible methods to achieve various results. Users are referred to other manuals or articles where detailed procedures are given.

While there is a recognized need to standardize procedures as much as possible, there is usually no concensus as to what these procedures should be. There is no single procedure that performs well under all conditions and for all parameters. Choices and compromises must therefore be made. Although this guide attempts to outline the steps in the planning process and the options available, the final choice and execution is the responsibility of individual investigators.

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1. INTRODUCTION

There is an increasing requirement for environmental information on arctic marine sediments because of natural resource development, particularly offshore hydrocarbon exploration and associated proposed transport mechanisms. At the same time, it is recognized that methods that have been used for collection, analysis and data reporting need improvement if the data are to have long term value (Thomas et al, 1982). Many of the shortcomings of past studies have been a result of a lack of awareness of the basic requirements of a sound environmental method and the limitations imposed by arctic conditions. Studies of chemical and biological sediment characteristics involve a number of steps from collection through to analysis. As in any data gathering sequence, the end result is only as valid as the weakest link in the chain of operations. In the Arctic, extreme and prolonged cold for much of the year, continuous ice cover over most regions for 8-12 months and the remoteness of the region from major population centres are all major obstacles to sample collection and analysis. As a result, sampling and field techniques have often been the weakest link in arctic marine environmental studies.

Government agencies and industries requested to provide environmental marine sediment data in support of development or to satisfy regulatory requirements are faced with a wide array of potential sampling and analytical methods from which to choose. Choosing the best or most appropriate methods can therefore be confusing and frustrating especially when there is a lack of consensus as to the best procedures and the requirements of individual situations. This guide attempts to outline the steps involved and to offer a number of choices to assist in the decision making and planning process.

1.1 Elements of an Environmental Method

The general elements of an environmental method as outlined by the ACS Committee on Environmental Improvement (Keith et al., 1983) are:

1) definition of goals;
2) preparation of a plan for sampling, analysis and reporting data;
3) sampling, subsampling and transport;
4) analysis;

5) methods of quality assurance;
6) reporting and documentation.

The goal needs to be clearly defined at the outset. This involves identifying the problem(s) and the information requirements and the use for which the data are intended. This could range from collection of preliminary information in an area where none exists to the more rigorous requirements of permit compliance or litigation. The goal needs to be clearly defined as ambiguity or too much generalization will make the goal impossible to achieve. Once the goal(s) has been established, a plan should be devised. The plan will define the population to be examined (area, extent, type of sediment, depth, species); the tests to be performed, desired performance characteristics (precision, accuracy, confidence limits); the methods for sampling, measurement and reporting given the performance requirements and intended use of the data; and finally, cost and time requirements. Definition of goals and planning are steps that must be undertaken by management or by a regulatory agency to satisfy the individual needs of different situations. The sequence of events in formulating a plan will undoubtedly be an iterative process. Goals usually require modification when faced with practical limitations. The plan must be modified if the chosen methods are not practical in terms of time, funding, logistics and facilities. After many iterations, it may become obvious that the goal cannot be achieved forcing either a modification in expectations or an increase in the allocation of resources.

1.2 Scope of the Guide

This guide deals with the development of a plan for meeting a specified objective and includes the following subjects:

- sampling strategy and means of quality control
- sampling and subsampling methods for chemical analysis
- sampling methods for benthos
- means of sample preservation and storage
- sampling logistics: transport; positioning; sampling from ice.

- methods of sample analysis for:
  - metals
  - organics:
    - TOC
    - oil and grease
    - petroleum related
    - phenols and chlorophenols
    - chlorinated hydrocarbons
  - grain size
  - Pb-210 dating
- methods of laboratory quality assurance and minimum requirements for reporting
- methods for the selection, collection and maintenance of organisms for bioassays
- methods for the analysis of benthic data

The following sections outline a process for selecting procedures within each of these subject areas.

2. HOW TO USE THE GUIDE

This guide is intended to aid in formulating a plan to meet specified objectives. However, users of this Guide will need to make decisions as to what methods best suit their objectives. Users must therefore provide answers to the following questions. These subjects are outside of the scope of the Guide:

- what are the goals/objectives of the study?
- when can sampling be undertaken?
- where is sampling to be undertaken?
- what level of accuracy is required or acceptable in the analyses?
- what is the time available and the approximate budget for the study?

Developing a plan will invariably be an iterative process as modifications and compromises will be required to stay within budget and allow for restrictions imposed by practical constraints in sampling and analyses. The overall plan will need to consider three areas:

1) **sampling** including where, when, how and how much to sample and sampling quality control measures;

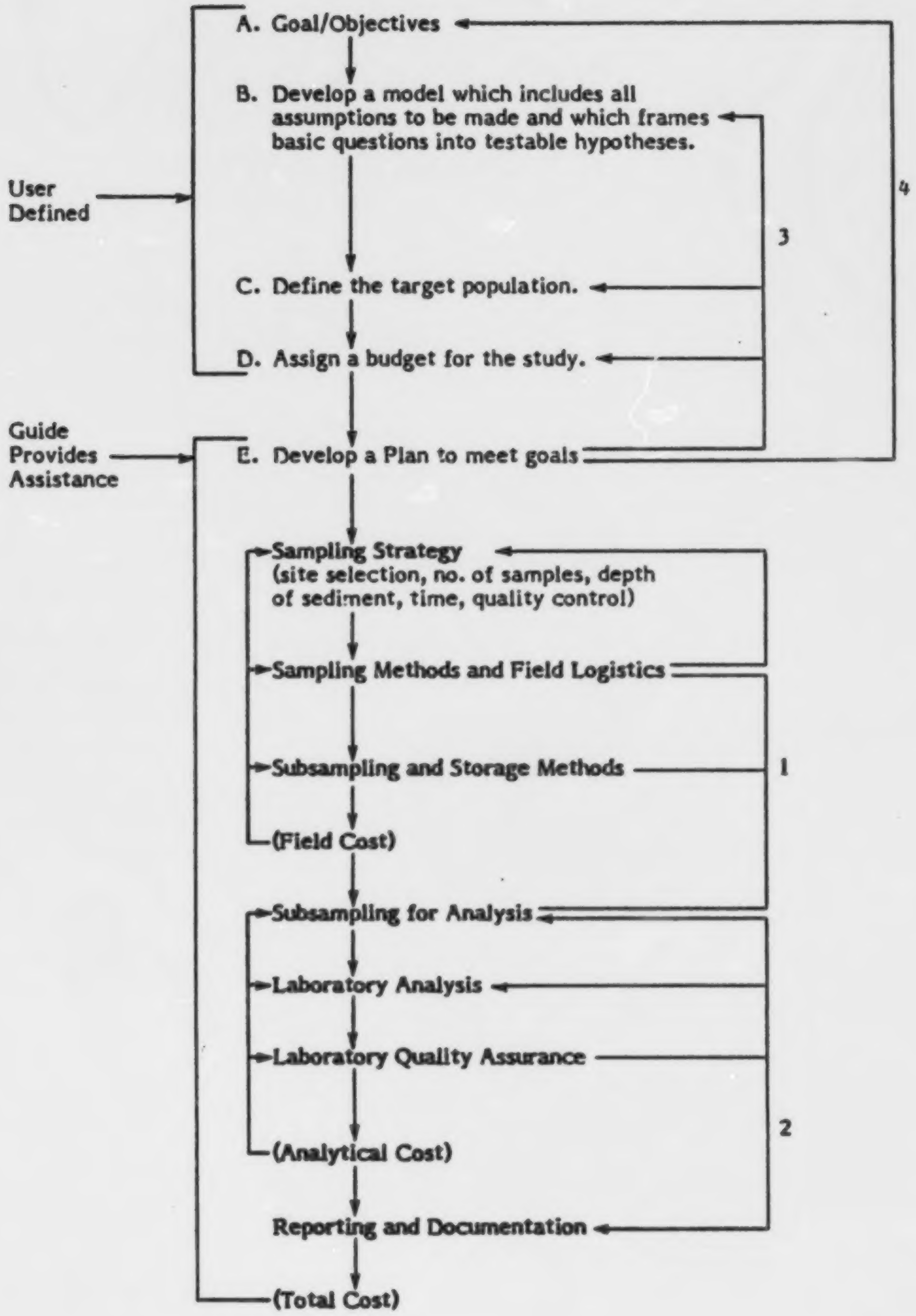
2) **analyses** including storage, preservation, digestion, analysis, and quality assurance; and

3) **reporting** of the data.

The sections of the Guide are organized in a sequence which takes the user from sampling, through analyses to reporting. The sections give a brief introduction and background. This is followed by a series of questions regarding goals and user requirements which lead to a final selection process. **Detailed procedures or descriptions of equipment and methods are not given.** Instead, users are referred to readily available references or existing manuals or guides for details.

Although sections are presented separately, it should be stressed that the final plan must encompass all aspects of sampling and analysis. It should also be pointed out that the order given in the guide need not necessarily be followed. The final plan must integrate the requirements of sampling, analysis and reporting and therefore a plan for each of these different aspects must consider the requirements and restrictions of the whole study. Definition of goals should include an assignment of priorities if there is more than a single objective. If for instance, the primary objective of a program is to obtain a certain level of sensitivity or precision in an analysis, this requirement may dictate that only one method is possible which will in turn limit the choice in other areas (e.g., the type of analytical method chosen will influence choice of sampler, sample size and means of preservation). Choosing an appropriate analytical method is often the most important step and will precede choice of sampler. If a primary objective is winter sampling, then sampling logistics and available samplers may be the critical factor and limit the types of analyses that can be undertaken. Finally, all decisions must be reconciled with the funding available. The interplay of various factors is illustrated in Figure 2.1.

A worked example is given as an Appendix for a hypothetical requirement and serves to illustrate some of the complex and often mutually exclusive requirements imposed by a broad based research or monitoring program.


A. Goal/Objectives
B. Develop a model which includes all assumptions to be made and which frames basic questions into testable hypotheses.
User Defined
4
3
C. Define the target population.
D. Assign a budget for the study.
Guide Provides Assistance
E. Develop a Plan to meet goals
Sampling Strategy (site selection, no. of samples, depth of sediment, time, quality control)
Sampling Methods and Field Logistics
Subsampling and Storage Methods
1
(Field Cost)
Subsampling for Analysis
Laboratory Analysis
Laboratory Quality Assurance
2
(Analytical Cost)
Reporting and Documentation
(Total Cost)

Notes:
1. Choice of analytical method will dictate sampling apparatus, sub-sampling methods, sample size and storage conditions.
2. Quality Assurance Program will influence the level of reporting and documentation required.
3. Limitations imposed by components of the plan may necessitate a revision to budget, scope and/or assumptions of model.
4. If a plan cannot be devised within the constraints of B, C or D, then the goals may have to be revised.

Figure 2.1 Flow Chart of Planning Process

3. SAMPLING METHODS FOR CHEMICAL ANALYSES

3.1 Sampling Strategy

The strategy adopted for the sampling program will depend on the goal. All goals can be classed in a general sense into one of two categories:

a) descriptive: goal is to describe the present sediment or biota characteristics of an area. Examples might be a preliminary survey for a more detailed analytical plan; background survey; or simply to determine whether a particular substance is present or detectable.

b) analytical: the goal is to compare the present conditions with those of another time or area to determine effects (impacts) or changes (monitoring). Procedurally, hypotheses are formed and tested.

The type of goal (analytical or descriptive) will influence decisions made regarding where to sample (study area), how many locations to sample, how many samples should be taken at each location and how sites are to be selected. These factors are very much site and study specific. To answer these questions, users must assemble as much information as possible regarding features of the study area (ice conditions, bathymetry, currents, ice scouring, water properties and influence of river input) and the characteristics of the contaminant(s) (typical levels, susceptibility to degradation, chemical forms) and species (abundance, species richness, seasonal variations in contaminant body burdens). This information can be used to develop a model of contaminant distribution and aid in the planning process (see Section 5).

The effects of weather, ice and restricted daylight present obstacles to any arctic sampling program. These factors will dictate or influence many important aspects of sampling including: when to sample, time required, transportation, equipment performance, clothing and shelter requirements. In preparing a sampling plan, it is essential to review the available information for the region of interest regarding expected weather, usual ice conditions and transport restrictions.

There are several overviews of climatic and ice conditions which can be consulted including the Pilot of Arctic Canada, Volume 1 (Canadian Hydrographic Service); Volumes 3A and 3B of the Beaufort Sea E.I.S. (Dome Petroleum et al., 1982), and texts such as Bryson and Hare (1974) and Hare and Thomas (1974).

Detailed weather observations for stations around the Arctic can be obtained from most weather offices of the Atmospheric Environment Service, Environment Canada, throughout Canada.

The sampling plan must allow for contingencies based on actual conditions encountered in the field. Equipment malfunctions, poor weather, ice conditions, logistic problems and sediment composition can all destroy the best formulated plan. "Down" time may more than double the time required to sample especially in short surveys. The field investigator may be forced to abandon the whole attempt or, in an effort to salvage something, may make a number of sampling decisions which may or may not satisfy the goal. **Consequently a "minimum" program should always be specified, and the goals clearly established so that changes can be made that will, as much as possible, be in harmony with the stated objectives.** In the end, the success of a sampling program may depend as much on the quality of field personnel and their ability to adapt to unforseen circumstances as to the thoroughness of the original plan.

> **When sediments are being collected for both analysis of chemical constituents and benthic populations, the needs and constraints of both requirements need to be considered and the necessary strategies and methods integrated into the final sampling plan. Section 5 should be consulted for a discussion of hypotheses formation and testing and sampling design.**

3.1.1 Definition of the Study Area

Boundaries to the study area must be established at the outset. Definition of the boundaries can be made in terms of:

- bathymetry and bottom topography (depth contours, basins, channels, etc.);
- texture of sediments;
- expected zone of influence of a contaminant input;
- practical constraints imposed by transport capability and the time and budget available;
- ice conditions

3.1.2 When to Sample

It is impossible or very difficult to sample in the fall during freeze-up and during ice break-up. Conditions are unstable and unpredictable in most arctic regions during these periods which may extend from mid-June to late July and from late September to mid-November depending on the region and local conditions. Some arctic regions will remain ice covered for a whole year. The optimum times for sampling in terms of weather and ice conditions are usually mid-August to mid-September (ice free period) and April and May (ice covered period). Most sediment surveys are carried out in the ice free season. However, when an objective is to determine contaminant body burdens in benthos, seasonal sampling should be undertaken as body burdens will vary with stages in the life cycle, health, reproductive stage and body weight of organisms. Weather records for the region and advice from local residents or persons who have worked in the area should be considered when planning the best times to sample.

3.1.3 Depth of Sediment to Sample

Depth of sediment required will be a function of the goals. However, it is **essential** in most sediment sampling for chemical analysis that the **surface layer be collected undisturbed.** In most studies, only **recent** or **present** concentrations of contaminants are important or required. Sedimentation rates throughout the Arctic will likely be 1 mm/year or less so that the top centimetre of material will represent at least 10 years of accumulation. The loss of a few millimetres of surface material therefore will mask any recent contaminant input.

Deeper sediments will be of interest in a number of situations. Dredging for instance may be to a depth of tens of metres and it may be necessary for permit applications to determine chemical constituents to the depth of expected dredging. Alternately, deeper sediments may be required or used to assess **"historical"** concentrations. This is an alternate means of providing a control in monitoring studies when the analytes are relatively stable (not easily degraded), especially when it is not practical to sample surface sediment at a control location because of time or distance constraints, or when no suitable site (i.e. same sediment characteristics but remote from zone of impact) can be found.

3.1.4 Where to Sample and How Many Samples to Collect

Choosing where to sample, how to select individual sites and how many samples to collect is perhaps the most difficult decision in the development of a sampling plan. Some guidelines are given below. However, the final decision will ultimately be a subjective one and may take many iterations to finalize. Users should also consult reviews by Kratochvil and Taylor, 1981; Green, 1979, Saila et al., 1978, Eberhardt et al, 1976.

3.1.4.1 Where to Sample

Assuming that the boundaries of the study area have been defined, it will be necessary to decide where within those boundaries to sample. Guidelines have been developed for dredging and ocean dumping related assessment. The EPA (Pequenat et al, 1981) recommends a minimum of six sites in conjunction with assessment of ocean dumping in the United States (two stations outside zone of influence - controls; two stations at the dump centre and two stations downstream or within the zone of influence). The Environmental Protection Service (EPS) in Canada should be consulted for guidance in sampling strategy in support of Ocean Dumping. In other instances, the number and location of sites must be determined on the basis of cost/site and location, goals and assumed spatial distribution of the contaminant and homogeneity of sediment within the study area. The final decision on number and location of sites must take into account the total number of samples as discussed in the next section.

The location of sites can be made on the basis of:

1) **previous site locations** in the case of a follow-up study;
2) **depositional environments.** If the goal of the study is to detect the presence of a specific constituent, then sites should be in regions of sediment accumulation. Shoals, steep slopes, sill crests and gravel beds should be excluded. **Basins and regions of fine textured sediment (mainly clay and silt) should be selected** as these are regions where contaminants will tend to accumulate;
3) **assumed or known current patterns, transport mechanisms, or deposition patterns.** For instance, Saila et al. (1978) found trace metals near a dump site in the New York Bight could be described by a log normal distribution, with metal concentrations decreasing

exponentially with distance from the dump centre. Saila et al. therefore proposed locating sample sites on a series of circles of logarithmically increasing size out from the dump centre. Positioning accuracy can be used as the criterion for determining the minimum circle diameter.

Once the potential sites have been identified it is usually necessary to choose only a portion to sample as budget and time constraints preclude sampling the whole study area in detail. There are basically two statistically defined means of selecting which sites to sample. Samples can be taken at random or in some sort of systematic way. The steps involved are:

1) The target sediment or biota is selected as outlined in Section 3.1.1 and 3.1.4.1 above. The target population (which may include the whole study area or just certain parts) is divided into imaginary units with diameters of twice the positioning accuracy or dimensions required by regulatory guidelines.

2) **For background, preliminary or descriptive surveys, or where no previous information is available, sites should be selected on a random basis.** This can be done with the aid of tables. Detailed instructions can be found in the text by Cochran (1977).

3) When the study area can be subdivided into smaller areas on the basis of physical characteristics or expected distributions (i.e. high or low probability of an impact), then a stratified system of selection can be employed. The area is first divided into strata and sites within each are selected randomly. **This is referred to as stratified random sampling and is discussed in detail in Cochran (1977).**

4) **Systematic selection of sites is a potentially more efficient means of site selection when there is background information available on the distribution of the analyte.** Systematic site selection is also applicable in determining zones of impact from a known point source of contamination. In systematic sampling, selection of the first site determines the remaining selection. Examples would be sampling at predetermined distances from a point source or in a defined spatial pattern (grid). See Cochran (1977) for details.

5) If the goal is to assess the impact of a discharge, then selection of at least one control site is important. See Section 5 for a discussion of control selection for various types of studies.

6) When currents are known, more sites should be selected in the direction of the predominant current direction.

3.1.4.2 Number of Samples

The number of samples collected will be the sum of the number of sites selected and number of samples required per site. Decisions regarding the total number of samples and how they are to be allocated among sites will be a function of:

- goals
- size of the study area
- degree of confidence required in the data and requirements of the quality assurance program
- heterogeneity of the sediment/biota distributions
- degree of segregation of the analyte in the sediment matrix
- cost (time)

Consider preliminary cost estimates: If a fixed budget is available for sampling, then an initial estimate of the cost to get a party into the field and return as well as the approximate cost of sampling per site should be made. If this is not done, then a lot of time can be wasted designing a sampling program only to find that there is time and money available to do only a fraction of it. This exercise will give a rough estimate of the time required and the number of sites that can be sampled. Section 4 (Logistics) should be consulted at this point as the logistic support required will dictate sampling expense.

When calculating the cost of sampling per site, it will be necessary to take into account the season and mode of transport. It is far more difficult (and time consuming) to sample at different sites in the winter or through ice. To move to a new site and sample in the winter or on the ice may require a whole day or as much as 5 times that required from a ship in open water. Transport requirements (to the study area and within the study area) can be costly. Ship charters can run from $ 2,000 - $10,000/day when available. Helicopter time will be in excess of $ 500/hr (see Section 4.3). These are fixed costs which must be taken into account in planning sampling expenditures.

Regardless of the goals however, certain minimum requirements can be assumed: **at least three sites should be sampled** (unless the sediments are known to be homogeneous) and **at least two and preferably three replicate samples should be taken at each site.** Three is the minimum number required to calculate a mean and standard deviation. **In comparative studies or when plotting spatial distribution from a source, control or background site(s) should be included.**[1]

Perhaps the most important factor influencing the number of samples to be taken beyond the minimum stated is the degree of sediment heterogeneity and segregation of analyte within the sediment matrix. When no previous data are available, these factors can only be assessed by undertaking a preliminary survey or by making assumptions based on the characteristics of adjacent areas.

The variance in the concentration of a given analyte can be defined on three scales:

i) variance over the study area as a whole, s^2_a

ii) variance within the boundaries of a particular sampling site or location, s^2_L, and

iii) variance within a single sample (grab or core), s_s^2

Generally, $s^2_a > s^2_L > s_s^2$ and the number of samples would therefore be allocated in the same order i.e. number of sites sampled would exceed the number of replicates at a given site which would be greater than the number of replicates from a single sample. Taking extra subsamples from a sample entails no extra sampling costs and is usually a required quality control measure. Subsampling variance s_s^2 however does need to be known as it will contribute to the uncertainty in sampling from a site (s^2_L) and from the area as a whole (s^2_a). Some possible situations are:

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[1] A means of economizing in the number of sites for some chemical parameters is to utilize a "historical" control. Deeper portions of a core sample at a site (below depth of expected bioturbation or other physical disturbances) will give a record of deposition over periods of a hundred years or more. **This type of control is only suitable, however, for analytes that do not undergo time related changes or degradation.**

If $s_a^2 \gg s_L^2$, then the emphasis in the program should be placed on sampling as many sites as possible.

If $s_a^2 \sim s_L^2$, then there should be a more or less equal allocation of samples between the number of sites and the number of replicates taken at a site.

If $s_a^2 < s_L^2$, then few sites should be sampled and the emphasis placed on obtaining more replicates at the sites chosen.

It should be stressed that the above relationships can only be used as a guide when there is sufficient background information available from previous studies or as a result of a preliminary survey. The confidence in the data will increase with increasing n (roughly as $n^{1/2}$). Analytical costs will increase roughly in direct proportion to n, while sampling costs will increase a negligible amount for extra replicates at a given site and by only a small fraction of the total for extra sites. **Therefore it is recommended that extra samples be collected.** Not all samples need be analyzed but the extra samples may help to clarify or affirm unusual results.

If the variance is known or can be estimated, the number of samples required to attain a given level of confidence in the data can be calculated (Kratochvil and Taylor, 1981):

i) where the analyte has a normal or Gaussian distribution (average greater than variance):

$$n = \frac{t^2 s^2}{R^2 \bar{X}^2}$$

where t is the student's t-table value, s^2 the variance, R the relative standard deviation acceptable in the average and $\bar{X}$ the mean.

ii) For substances or analytes whose concentrations have a Poisson distribution in the sediment (randomly distributed such that the variance is approximately equal to the average):

$$n = \frac{t^2}{R^2 \bar{X}}$$

iii) For substances with a negative binomial distribution (analyte occurs randomly in patches or clumps - variance is larger than the average)

$$n = \frac{t^2}{R^2} \left(\frac{1}{\bar{X}} + \frac{1}{K} \right)$$

where K is called the index of clumping (and must be estimated from preliminary data).

Conversely, the confidence limits for the data can be calculated afterwards on the basis of n and the observed variance and mean.

Visman's "General Sampling Theory" (see Ingamells, 1974) can also be used as a guide to choosing the number of samples to collect when sampling inhomogeneous and segregated sediment. It can also be used to assess the degree of inhomogeneity and segregation. Procedurally, two series of samples are collected, one using small sample weights, the other with large sample weights. Visman sampling constants can then be calculated on the basis of the results as:

$$A = w_1 w_2 (s_1^2 - s_2^2) / (w_2 - w_1)$$

$$B = s_2^2 - A/w_2$$

where A is a homoegeneity constant, B is a segregation constant, s_1^2 and s_2^2 is small and large sample variance respectively and w_1 and w_2 are the small and large sample weight.

Once A and B have been determined, the expected variance for N samples each of a weight w is given by:

$$s^2 = A/W + B/N$$

where W = Nw, the total weight of all samples.

3.1.4.3 Composite Samples

A practice sometimes carried out is to pool a number of samples, homogenize them, and then analyze subsamples of the composite. This approach will yield an average compositional value and can provide a substantial cost savings as fewer analyses will be required. Although the average of a properly prepared composite and that of a number of individual analyses will be the same, the between sample variability is lost. Since this is usually a very important statistic, **the use of composite samples is not recommended** unless it will satisfy the goals of the study. Composite samples are often a necessity however when analyzing benthic organisms for contaminants, since a minimum weight of material is required.

3.1.5 Matching the Sampling Plan to Available Funds

Once a plan has been devised, a more detailed cost estimate must be made. If the estimate exceeds the allocated funding, then obviously revisions will have to be made to the plan. One solution is to collect the number of samples as planned but to analyze only a percentage of the collected samples (i.e. cut analytical costs). The steps taken must be reconciled within the framework of the overall goals. However, many revisions to the plan may be required to finally match the sampling plan with available funds. It is wise in estimating sampling costs to include a contingency of as much as 50% of the estimate (depending on the length of the study) for delays and unforeseen circumstances, such as equipment malfunctions and bad weather and ice conditions.

3.1.6 Sampling Strategies for Detecting the Presence of Ore Concentrates or Drilling Fluids

Offshore hydrocarbon development and mining activities are currently the two main industrial activities producing wastes which are discharged directly to Canadian arctic marine areas. Drilling fluids and drill cuttings from offshore hydrocarbon exploration, primarily in the Beaufort Sea, are the major waste discharged; ore concentrates, entering coastal areas near marine loading terminals at Nanisivik N.W.T. and Little Cornwallis Island, N.W.T., have a smaller influence in terms of total amount of material discharged and the number and frequency of point sources. The sampling and analysis of marine sediment (or biota) to detect the presence of either drilling fluids or ore concentrates requires a knowledge of the chemical characteristics of the material, size distribution of the particulates, volumes of material discharged or lost and the location of the sources. A sampling survey to detect an industrial input is an analytical exercise as comparisons and hypotheses testing would be required.

Suitable tracers of drilling fluids in marine sediments will depend in part on the carrier fluid. Water-base muds and cuttings are routinely discharged to the sea. Oil-base muds are not, at the present time, routinely used in offshore drilling operations in the Canadian Arctic. However, guidelines for their use are presently being developed.

Some potential tracers of drilling muds/cuttings are listed below:

Water-Base Muds	**Oil-Base Muds**
barium	barium
chromium (when chrome lignosulphonates are used)	synthetic organics (Biocides)
synthetic organics (biocides)	aromatics and aliphatic hydrocarbons associated with base oils

Barium is probably the single most useful tracer because (1) it is abundant in drilling fluids; (2) it usually behaves conservatively in marine sediments; and (3) there are no other significant anthropogenic inputs of Ba to arctic marine sediments.

Ore concentrate from existing coastal mining operations in the Arctic, at Nanisivik and Little Cornwallis Island, can be distinguished on the basis of metal content and particle size. Concentrates are primarily lead and zinc; the most abundant metallic impurity is cadmium (Thomas et al., 1984)

Specifically, strategies for detecting waste drilling fluids and cuttings and ore concentrates in marine sediments will involve the following:

1. **mass balance calculations and selection of suitable tracers; consideration of receiving environment concentrations and oceanographic conditions including dispersal mechanisms.**

The first step is the mass balance calculation. This involves predicting accumulation rates of contaminants in the sediments by taking into account the rate of waste discharge, physical characteristics of the waste and the concentration of significant contaminants in the discharge. The importance of this step should not be underestimated. It will provide a first approximation of the scale of the disturbance and define the detection limits required to resolve the contaminant inputs against natural (background) levels. Another benefit of having an approximate indication of the scale of disturbance is that this information can influence the frequency of sampling and the distance between sampling points.

The natural (background) concentrations of those elements or compounds which are also tracers of the waste discharges will influence the monitoring strategy by setting detection limits. As the natural concentration of a constituent decreases in the receiving environment, it becomes progressively easier to resolve the constituent associated with the discharge source from the background, assuming that detection limits are sufficiently low.

Finally, oceanographic conditions (water depth, water column density structure, currents, tides, water column particulate load, frequency and duration of high energy events, and bedform movements) indicate the likely direction of waste migration, and the rate of dispersion in both the water column and sediments. Such information will help define the requirements for sampling equipment, sample replication, sampling frequency, and station locations (offshore drilling rigs routinely collect site specific oceanographic observations).

2. **modelling the dispersion of the drilling fluids or concentrate (assumed means of dispersion, oceanographic features) to provide a framework for predicting sediment distributions.**

Saila et al. (1978) found for instance that dispersion of heavy metals from a dredge spoil dump site followed a log-normal distribution. Thomas et al. (1983) predicted an approximate log-normal dispersion of metals associated with drilling fluid disposal from offshore platforms in the Beaufort Sea. The same study predicted a bimodal distribution in Scotian Shelf sediments as a result of the increased tidal influence in dispersion.

3. **using a systematic sampling scheme to choose sampling sites with spacing based on the assumed dispersion model.** For a point source, some sort of radial plan centred on the source is probably most appropriate (Saila et al., 1978; Crippen et al., 1980) in an open sea area with no physical boundaries (see Section 5).

4. **sampling and subsampling must be designed to recover the top cm of sediment undisturbed as this will contain recent input (see Section 3.2).**

5. **if the wastes have a well defined particle size range, the sediment should be fractionated according to size and only the appropriate size range analysed to improve sensitivity.**

3.2 Choosing a Sampler for Chemical Analysis

3.2.1 Introduction

Sediment samples for chemical analysis can be collected either remotely from a vessel or through the ice, or directly by means of divers or submersibles. Remote sampling is the most common means of collecting sediment and, in most instances, the only practical one. However, direct collection using divers or submersibles, though more costly and difficult, offers distinct advantages.

There are two basic remote sampling devices which can be recommended for use in the collection of sediment samples from a surface platform: 1) grabs which take a "bite" of surface sediment; and 2) corers which collect a column of sediment.

The ideal remote sampling device for chemical analyses is one that can obtain and retrieve an undisturbed sample in sufficient quantity for all analytical requirements. The sampler should be easy to use, not contaminate the sample, have a reasonable cost and be effective for use over a wide range of sediment types. No one sampler satisfies all these requirements; sampler selection, therefore, involves a number of compromises based on the overall goals of the program, the type of sediment and whether or not depth profiles are required. The term "undisturbed" when applied to sediment sampling is a relative one. In most instances it implies that the sample has not been compacted, mixed or had loose surface material displaced. There is, however, no absolute measure of disturbance as the original sediment characteristics are not known and all samplers, of necessity, disturb the sediment where the cutting edge or body of the sampler enters the sediment. Significant disturbance may not be visually obvious or apparent from analytical results. Subsequent data may seem plausible but be in error due to sample loss (Baxter et al., 1981). The degree of disturbance that can be tolerated will vary with the intended use of the data. If the goal is to obtain a bulk sediment, mixing of the sediment will be of little consequence. Where depth profiles are needed, however, mixing and compaction will lead to erroneous results. **Loss of the surface fines can not be tolerated if the effects of a recent input are required.**

Although there have been no definitive studies undertaken, the design features of samplers that limit sample disturbance are:

a) speed on entry: for the same sampler the degree of disturbance decreases with speed of entry.

b) surface area: the larger the open vented surface area relative to the wall thickness, the less disturbance.

c) cutting mechanism: the cleaner and faster the cutting action, the less disturbance due to mixing.

d) sampler wall thickness: thinner walls give a "cleaner" cut.

Positive contamination of sediments from samplers can arise from introduction of analyte or interfering analyte from the sampler itself, from the operation or lowering wire and from mixing of sediment from different depth horizons. The number of materials available for sampler use is limited since samplers have to be fairly robust. Almost all sampler components are metallic. As in the case of surface disturbance, there are no studies that might indicate how important sampler materials are or how severe contamination effects might be. Steps to limit sampler contamination are based on common sense and caution. Obvious features like flaking metal or paint, rust or corrosion, oil or grease, must be avoided. As a precaution, sediment for metal or organic analyses are always taken away from the sampler body. Plastic liners and core barrels are available for gravity corers and although preferable for metals, are a possible source of contamination for some hydrocarbons. Preferred materials for hydrocarbons are stainless steel and anodized aluminum. It is possible to have samplers Teflon coated, but there are no data to indicate whether this is a necessary precaution.

Sample handling is an often overlooked potential source of error. There is not the same sense of caution that is usually applied to a relatively "clean" process like water sampling. However, care must be taken that clothing, tools, lines, winches and hands do not become sources of contamination.

Beside factors which influence sample integrity, there are a number of other sampler features that are important from a user's standpoint. Obvious items are bulk and weight which will dictate lifting capacity required, ship requirements and ice hole size. More subjective factors are ease of operation and reliability. These latter aspects of sampler design are often crucial to the success of a sampling programme, especially in adverse weather conditions.

Except for very light grabs in shallow water, most remote samplers require a winch to retrieve the sampler and sediment. Lifting capacity and power requirements are important considerations. Approximate lifting requirements for various types of samplers are shown in Table 3.2.1. Also, as water becomes deeper it may be more difficult to tell when the sampler is on the bottom and a pinger may be required so that excess line is not played out which may subsequently tangle on the sampler (U.S. Naval Oceanographic Office, 1968).

Grabs and corers are different in design, and the two types of devices have very different applications. Grabs are necessary where a relatively large area (or volume) of sediment is required (i.e., for benthic sampling), and limited depth penetration is acceptable. Corers are required when depth profiles of more than about 15-20 cm are needed. Either type of sampler can be used to sample the upper 15 cm of unconsolidated soft sediments when limited quantities of sediment are required for analysis. Choice of sampler will depend on objectives, convenience and operational characteristics. Most grabs are limited by the depth of water in which they can be effectively used as their high surface area/weight ratio and shape can cause planing on descent (Bouma, 1969).

All remote sampling devices were designed for use from a vessel. Some designs require a large lifting capacity and are very bulky. Many of these devices are, therefore, not practical for through-the-ice sampling where size becomes a limiting factor.

3.2.2 Recommendations

Recommendations for sampling devices for sampling to various depths and from different platforms are given in Table 3.2.2. Further details are given below.

(a) Grab Samplers

Features of recommended grab samplers are given in Table 3.2.3. Of the recommended grab samplers, the Ponar is perhaps the most versatile for collecting samples for chemical analysis. It is relatively compact and yet heavy enough to be effective in a range of sediments. The Smith-McIntyre is the largest and heaviest sampler and will, therefore, be restricted primarily to shipboard use. Its frame imparts good stability however, and it is therefore, effective on a wide variety of bottoms and slopes. A modification of the Van Veen incorporating a frame and slowed entry to minimize surface disturbance has also been produced (Meek and Ray,

TABLE 3.2.1

LIFTING REQUIREMENTS FOR VARIOUS TYPES OF REMOTE SAMPLERS

(from Moore and Heath, 1978)

SAMPLER TYPE	REQUIRED LIFTING CAPACITY (kg)
Grab samplers	
Birge Ekman:	25-50
Ponar, Van-Veen	110 - 250
Smith McIntyre:	200 - 300
Gravity Corers:	
Open- barrel	200 - 2500
Phleger	70 - 100
Multiple (3 - 5 barrels)	250 - 600
Piston	200 - 2500
Box	350-400

Table 3.2.2

Recommended Samplers for Chemical Analysis

Depth of Sediments Required	Through the Ice Sampling		Open Water/Large Boat Research Vessel		Open Water/Small Boat or Aircraft on Floats	
	Type of Sampler	Comments	Type of Sampler	Comments	Type of of Sampler	Comments
a) < 15 cm	Birge Ekman	Will fit through 30 cm hole; only suitable for soft bottoms in shallow (30-50 m) water;[1]	Same as ice plus Smith-McIntyre Grab	Method of choice when a large quantity (> 500 g) of sediment required.	Same considerations as for ice sampling	
	Gravity Corer: (cylindrical; phleger, KB, benthos type). barrel I.D. 6-10 cm.	Will fit through small hole; difficult to retrieve the top few cm undisturbed; core catchers must not be used. A winch and support over the the ice hole will be required.	Craib type hydraulically damped corer	One of best samplers available for collecting undisturbed cores; capable of sampling sandy substrates; small diameter barrel; therefore limited sample; frame makes the sampler very bulky.		
	Multiple corer:	Requires a larger ice hole, but offers the advantage of collecting several cores at one time; this is extremely useful for assessing substrate homogeneity and reduces the sampling time	Box corers	Generally regarded as best samplers available for obtaining undisturbed sediment. Has a large surface area. This is the sampler of choice when a lot of sediment is required and sufficient lifting capacity available: (>500 kg); drawbacks are bulk and complexity.		
	Diving	Require large ice hole plus support equipment. Restricted to shallow (< 20m) water. Expensive; However, best method for ensuring that undisturbed sediment is collected. Allows for a great deal of flexibility in site location.				
	Compact Van Veen/Ponar	Requires larger hole than Birge Ekman; surface fines more likely to be lost; -can be used in deeper water -often used in benthos studies				
		Van Veen sampler of choice for combined benthos/chemistry studies.				
b) >15-50 cm (approximately)	cylindrical gravity corer: e.g., Benthos, Phleger, KB, etc.), extra weights required	Will fit through small hole in the ice; surface fines may be lost. Some compaction may occur as speed of entry will have to be greater to achieve sufficient penetration; winch and some sort of support over the ice hole will be required.	As for through ice sampling plus: Box corers	Comments as per (a) extra weights/lifting capacity likely required. Box corers are the best method for collecting undisturbed cores to this depth.	Same considerations as sampling from the ice; the requirement for a winch and davit or boom may make sampling to this depth impossible from a very small boat.	
	Diving	30-40 cm cores are probably the limit to which a diver operated corer can penetrate in sediment and be subsequently removed without assistance from the surface.				
c) > 50 cm	Generally not practical without drilling a large hole and without considerable lifting capacity (500 kg or more) to accommodate larger samplers. Only really practical to sample to this depth in fairly soft unconsolidated sediment.		As for ice sampling; in very soft sediments, simple gravity driven cylindrical or box corers may penetrate to 100 cm or more. If greater depth is required, a piston-type corer will be required; Sampling this depth of sediment will usually require two methods with a separate sampler being used to retrieve the upper few cm.		Not feasible unless bottom sediments extremely soft; (see comments for through the ice sampling).	

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1) probably the only sampler that can be retrieved easily by hand.

TABLE 3.2.3

FEATURES OF RECOMMENDED GRAB SAMPLERS

TYPE	WEIGHT (kg)	AREA SAMPLED (cm^2)	MAX. DEPTH OF PENETRATION (cm)	SURFACE DISTURBANCE (relative)	PERFORMANCE IN VARIOUS SEDIMENT TYPES				SUSCEPTABILITY TO SEDIMENT WASHOUT	COMMENTS	REFERENCES
					SOFT CLAY, SILT	SAND	GRAVEL PRESENT	HARD CLAY			
Van Veen	19 - 41	- 2000	20 - 30	low-medium depending on size of screen openings	good	good	sometimes	sometimes	low, except in coarse sediment or if shells, debris present which will prevent jaws closing completely	modified Petrsen grab with long scissors-like arms which improve efficiency and penetration; short arms have been used for through-ice sampling	Mawhinney and Bisutti, Petersen, 1977
Modified Van Veen (Sontar-Van Veen)	- 80	1000	20 - 30	low					as above	mounted in a frame; sampler slides slowly down guides after frame hits the bottom; very stable; minimal disturbance; has been deployed by a helicopter in support of studies close to an offshore drilling rig	Meek and Ray, 1980
Ponar	23 - 30	400 - 550	20	low	good	good	sometimes	sometimes	low, overlapped jaws and side plates; washout may occur when gravel prevents complete closure	good all-round samplers suitable for most sediment types: jaw shape unlike van Veen, Petersen exactly follows arc of cut minimising sample displacement	Plumb, 1981 Mawhinney and Bisutti, 1981
Smith-McIntyre	45 - 90	1000	20	low	good	good	sometimes	sometimes	low, as for van Veen	basically a van Veen sampler mounted on a frame. Has trigger plates in frame so that open grab is pushed into sediment with springs (force > 35 kg); very stable because of frame; able to sample on slopes; could be deployed by a helicopter as for Souter-Van Veen	Smith-McIntyre, 1954
Birge-Ekman	5 - 10	250 - 500	10 - 20	low	good	sometimes	no	no	low, as for Petersen	poor stability in all but calm conditions, soft sediment ideal for through-ice sampling because of its compact size. Messenger triggered; spring activated jaws. Jaw shape exactly follows arc of cut minimizing sample displacement	Howmiller, 1972

1980). The main drawback of the Birge-Ekman sampler is its light weight with the result that it is ineffective on hard bottoms and is more susceptible to planing on descent. It can therefore, only be used effectively in shallow water with weak currents and relatively soft, flat bottoms. Extra weights can be added to improve performance. The Birge-Ekman is the grab sampler of choice for through ice sampling in shallow regions with soft bottoms because of its compact size and excellent operational characteristics in these conditions. **The Van Veen and Smith-McIntyre (see Section 5) are recommended for benthic studies and are therefore samplers of choice when both benthic and chemical sampling are required.**

The cost of commercially available grab samplers ranges from $1,000 to $6,000. The smaller, simpler models such as the Ponar and Ekman are in the lower price range while the Smith-McIntyre and more elaborate Van Veen modifications will be in the upper price range. A partial list of suppliers and manufacturers is given in Table 3.2.6.

(b) Cylindrical Corers

Features of recommended cylindrical gravity corers are summarized in Table 3.2.4. Cylindrical corer features that are desirable and should be considered in choosing a particular sampler are:

1) mode of penetration: corers that can penetrate to a sufficient depth at reduced entry speed are preferable.

2) diameter of core barrel: larger diameter cores are desirable because of the need for sufficient sediment for subsequent analyses, because of less disturbance of the core as a whole and because contamination from the corer will be less significant. Walton (1978) recommended a minimum of 6 cm I.D.. Wall thickness should be the minimum needed for the expected impact force.

3) weight and lifting requirements: corers with variable weights (i.e., weight can be added or removed) are desirable because the corer weight can be adjusted for a particular sediment or desired depth of penetration.

TABLE 3.2.4

FEATURES OF COMMON CYLINDRICAL GRAVITY CORERS

EXAMPLES	APPROXIMATE WEIGHT (kg)	DIMENSIONS	SURFACE DISTURBANCE (relative)	DESIGN FEATURES	SEDIMENT TYPES	COMMENTS	REFERENCES (See Table 3.2.6)
Phleger, Alpine	28-45	variable: core barrels usually small diameter (4 cm)	moderate	very simple design; no moving parts: uses a one-way valve system on top. Water pressure forces this up on descent. Most models have a fixed weight (e.g. Alpine); some have variable weights and stabilizer fins.	soft, sandy, semi-compacted: not a good choice for coarse-grained sediment	commercially available from several suppliers: all are steel construction with metal barrels: plastic liners and core catchers available.	Kahl Scientific Mawhinney & Bisutti, Bouma, 1969. Sly, 1969
custom-designed Phleger-type:	7 without weight. up to 7 kg per each lead weight	variable:	low-moderate (decreases as diameter of core barrel increases)	Usually aluminum frame, stainless steel cutting head; clear acrylic core barrels: variable lead ring weights; one-way valve consists of rubber bung. No stabilizer fins.	as above	easily constructed	Mawhinney & Bisutti, 1981 Sly, 1969
Benthos	up to 70	core barrel 6.6 cm: overall corer diameter ~25 cm	low-moderate	reliable design: spring activated flapper valve; stabilizer fins.	soft, semi-compacted or compacted fine-grained: not a good choice when gravel present or in sand	commercially available	Benthos Corp.
Triple Corer or multiple corer	28 (steel tubes) 21.5 (acrylic)	three (or more) core barrels: dimensions as above	low-moderate	stainless steel pipe or acrylic tubes.	clays, muds, silts	in-house modification	Kemp et al. 1971 Moore and Heath, 1978
KB^{TM} core	8 or 18 without core barrel	5 cm barrrel: overall: ~15-20	reported to be low: depends on speed of entry	has fixed weight and a messenger-activated valve closure: that does not require water pressure to keep it open.	soft sediments	commercially available; stabilizing fin extra weight available options.	Wildlife Supply Company
Craib (hydraulically damped)	44	5.7 cm diameter core barrel	low	hydraulic damper and ball closing device at bottom for sandy sediment: corer is mounted within a frame	soft sediments as well as sand	hydraulically damped; cores are 10-15 cm long	Craib, 1965 Baxter et al., 1981

4) materials: stainless cutting heads are best; clear acrylic core barrels have the advantage that the core can be inspected after retrieval without removing it. Plastic liners are useful features as they reduce contamination for trace metals and speed up corer use.

5) stabilizer fins are useful for deep water or areas with high currents assuring that the corer will strike the bottom in a vertical position.

6) core catchers ensure a higher success rate in the retrieval of cores but can cause considerable disturbance and mixing of the sediment. **They should not be used unless a sample cannot be retrieved otherwise.** A good seal on the end of the barrel is usually sufficient to "hold" the sediment in the barrel. Valve systems that seal tightly are, therefore, desirable.

The cost of cylindrical gravity corers varies directly with complexity. The simplest gravity corers can be built or purchased for under $1,000, while a corer with a frame such as the Craib will cost in excess of $5,000.

(c) Box Corers

Box corers are all gravity corers with large cross-sectional area (either rectangular or square) and limited depth penetration. Because of the large sufrace area, these samplers are the best available for collecting undisturbed cores and sampling at the sediment-water interface. The large area also means a larger amount of sediment is available for a given sediment horizon while the shape of the barrel allows a side to be removed facilitating core examination and sub-sampling.

Standard boxes are fabricated from steel or anodized aluminum. Karl (1976) has developed a plastic liner which reduces potential metal contamination and speeds up sampling. Most box corers require a frame for support as the samplers are top-heavy and very unstable. All corers use some sort of shovel seal for the bottom of the box to prevent loss of sediment on retrieval. This feature allows samples to be retrieved even in very loose, unconsolidated sediment and does not affect the integrity of the core. As with grab samples, these shovels may not seal properly because of rocks, debris or shells.

The major drawbacks of box samplers are the limited depth penetration, large size and higher cost (commercial models cost between $5-10,000). The bulk of this type of sampler prevents it being of use in through-ice sampling. Features of several box corers are compared in Table 3.2.5. A partial list of suppliers and manufacturers of corers is given in Table 3.2.6.

(d) **Diving**

Diving, when possible, is perhaps the best method of collecting an undisturbed sample. However because of the much higher cost, (see Section II - 4, Volume I) diving can only be justified when combined with a benthos sampling program and when depth (<20m) and visibility permit. The advantages to diver taken samples are:

1) suitable bottom area for sample collection can be selected if a particular sediment type is required.

2) corer or grab samplers can be operated to ensure minimal disruption of the surface layer, adequate penetration into the sediment, and proper closure of the sampling device; and,

3) visual observations or photographs of sediment conditions (such as ripple marks, ice gouges, slumping, etc.) can be taken to aid in interpretation of particle size and chemical analyses.

Diver-assisted sediment sampling devices suitable for chemical studies include the Ekman and Birge-Ekman grabs, simple corers, and core tubes or boxes which can be sealed at both ends (e.g., Mawhinney and Bisutti).

3.2.3 Reliability

It should be stressed that no one sampling device will successfully collect samples from a full range of sediment types. While grabs and corers are generally reliable and robust, a back-up sampler should be included in the sampling plan in the event the primary sampler becomes damaged or fails to collect a sample because of bottom features (stones, debris). A contingency for these events should be included.

TABLE 3.2.5

FEATURES OF BOX CORERS

REFERENCE	DIMENSIONS OF BOX	WEIGHT (kg)	FRAME DIMENSIONS	BOX MATERIAL	OPERATIONAL FEATURES
Bruland, 1974	20 x 20 cm 130 cm high	350 (up to 280 kg of weights can be added)	200 cm x 100 cm	anodized aluminum	uses two overlapping shovels to seal bottom of box: spring loaded plate seal at top of the box shut on retrieval, open on descent
Bouma and Marshall (1964) Bouma, 1969	20 x 30 cm 45 cm high	∽ 500 (320 kg of weight)	150 cm x 150 cm	3mm stainless steel	box open at top; single levered shovel seals bottom of box on retrieval
Texas A & M Sampler (cited in Bouma, 1969)	30 x 30 cm 91 cm high	> 600 (filled boxes weigh over 180 kg)	trapezium 120 cm on longest edge	4.7mm stainless steel	box open at top; improved versions of above sampler
Kogler, 1963 (cited in Bouma, 1969)	15 x 15 cm lengths of 1 - 4 m	can use up to 14 weights, 50 kg each	none	steel, PVC coated on interior	one way valve on top of weight stand, core nose has internal spring activated plates to prevent loss of core
Kahl Scientific Bulletin WAB-1464/1	10 x 25 cm 30 cm high	210 (with weights)	120 x 65	stainless steel	box has removeable side wall: box is sealed on top on retrieval with a flexible, thick rubber sheet

Table 3.2.6

PARTIAL LIST OF NORTH AMERICAN MANUFACTURERS OF CORERS AND GRAB SAMPLERS

1. Kahl Scientific Instrument Corp.
   P.O. Box 1166
   El Cajon, California 92022
   (all types of corers and grab samplers)

2. Benthos Inc.
   North Falmouth, Massachusetts 02556
   (gravity, piston and free-fall corers)

3. Wildlife Supply co.
   301 Cass Street
   Saginau, Michigan 48602
   (gravity corers, Eckman and Ponar grabs)

4. Hydro-Products
   11777 Sorrento Valley Road
   San Diego, California 92121
   (grabs, gravity corers)

5. TSK. Co. ltd.
   15109 - 30th Avenue. S.E.
   Bothell, Washington 98011
   (grabs, gravity corers)

6. Horizon Ecology Company
   7435 North oak Park Ave.
   Chicago, Illinois 60648
   (small grabs, gravity corers)

7. Intersea Research Corporation
   Post Office Box 2389
   La Jolla, California 92037
   (gravity corers, grabs)

8. Ocean Instruments
   5312 Banks Street
   San Diego, California 92110
   (box corers, Soutar-Van Veen grab)

3.3 Subsampling

Subsampling must be done in the context of:

i) type and number of analyses to be performed (dictates the minimum amount of sample required and the precautions involved).

ii) type of sampling device.

In terms of analyses to be performed, sections 6 - 10 should be consulted, sample requirements noted and special handling requirements and types of suitable storage containers determined.

General guidelines for sub-sampling are given below:

1) Immediately after retrieval, core barrels should be stoppered or jaws of grab samplers secured to prevent accidental loss of sample.

2) Sampler must be kept upright.

3) Surface water must be carefully removed (leaving surface fines undisturbed). If the water is extremely murky, surface disturbance will have occurred and fines must be allowed to settle, water filtered or a new core collected. This is perhaps the most critical step except when only bulk sediment properties are required (Walton, 1978).

4) Before sub-sampling, appearance of the core or grab should be noted, along with any obvious features (sediment type, colour, structures, fauna, odour). Any structures such as wood chips, large stones, etc., should be removed or not sampled.

5) For grabs where only bulk (non-depth related) analyses are required, the contents can be dumped onto a board or plastic sheet and sub-sampled according to Walton (1978). For depth related features, a small core can be taken from the interior part of the grab through the hinged tops. Sediment near the grab walls should be avoided.

6) For cylindrical cores, the complete core can either be frozen for sub-sampling in the laboratory or sub-sampled after collection. Some type of piston extruding device will be required for immediate sub-sampling (Kemp *et al.*, 1971). Frozen cores can be thawed slightly and extruded intact then cut with a saw. Alternatively cores may be first sectioned longitudinally (Walton, 1978; Bouma, 1969), depending on the number and types of analyses.

7) Box cores can be sub-sampled by removing one side (Bouma, 1969), taking cylindrical corers in the horizontal plane throuh pre-cut cylindrical holes at set depths in the box wall or by taking small cores vertically from the surface. Sub-sampling in the horizontal plane is only recommended in very loose, high water content sediments which can be extracted easily. A large diameter cut-off syringe with the rubber plunger replaced with teflon, or metal, is a convenient minature piston coring apparatus.

8) Utensils/apparatus for sub-sampling must be of a suitable material for the type of analyses to be carried out. For trace metals, plastic scoops or core tubes should be used. For organics, metal (stainless steel, aluminum) or Teflon materials should be used. Utensils used for sub-sampling must be kept in a clean container when not in use. Some cleaning solution (solvent or dilute acid) should be available to clean utensils if they are accidently dropped or exposed to contamination (see individual sections for each analyte).

9) As much sample as possible or practical should be stored for analysis. Minimum reqiurements must be known (see Note 1).

10) The order in which sub-samples are withdrawn should be strictly adhered to and reported. Samples to be analyzed for trace constituents (metals, organics) must be taken *first*; samples for grain size can be taken last.

Note: 1. **Analyses are usually performed on a dry weight basis.** Water content of sediment may vary from 30 - 80%. The amount of wet sediment collected should therefore be at least 2 - 3 times the dry weight of sediment required for the number of analyses to allow for water loss and for losses during drying, grinding and sieving.

3.3.1 Storage and Preservation

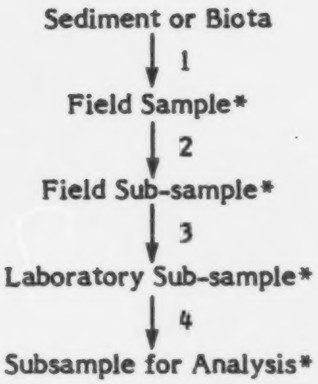
The topic of storage and preservation is discussed in Sections 6-10 for the different analytical methods. Specific requirements are given and cleaning procedures indicated. In general, the following guidelines apply for types of storage containers and preservation conditions:

TYPE OF ANALYSIS	TYPE OF STORAGE CONTAINER	STORAGE CONDITIONS
Metals:	plastic (polyethylene, polypropylene, teflon,) either bags or jars.	frozen
Organics: (all classes)	metal (stainless steel, aluminum), teflon; glass.	frozen
Grain size:	any of the above.	frozen or thawed

Note: **Sample containers must be robust:** glass containers need special packaging to prevent breakage in transport and must not be overfilled to prevent breakage during freezing. Most plastic containers become brittle at very low temperatures (-30°C or less) and will shatter or break if dropped.

3.4 Quality Control in Sampling

The steps involved in the sampling and subsequent reduction of a sample to a laboratory size suitable for analysis are indicated below.



Each of the above steps is a potential source of error. Steps 1 and 2 are carried out in the field and how well they are carried out is crucial to the reliability of the subsequent data. Procedures need to be incorporated into the sampling plan to provide quality control and to assess the magnitude of the error in the sampling and sub-sampling procedures. The topic of quality control in the laboratory is discussed in section 10. The goal of quality control measures in sampling are to control sources of error and assess the sampling and storage variability. It is not possible to measure the accuracy of the sampling as the "true" value and composition of a sediment are never known beforehand. Instead, measures of precision obtained by examining distributions generated if a procedure is applied repeatedly to the same population are used. Some elements of quality assurance applicable to sampling are listed below.

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*Note: There is an important distinction between a "sample" and a "sub-sample" (Ingamells, 1974) A single sample of sediment will rarely if ever be representative of the sediment or biota as a whole. The composition of the sediment can only be determined by taking a very large number of samples (i.e., step 1 must be done repeatedly). A sub-sample however, is usually considered to be a representative split or portion of a sample (sub-samples from the same sample should have the same composition). This distinction is important when considering quality control measures and in designing a sampling plan. The most replication should be concentrated in Step 1 (see Section 3.1.3.2).

1) **Replicate Samples:** At randomly selected sites, more than one sample is collected (includes variability due to sampling equipment, subsampling procedures and substrate heterogeneity).

2) **Split Samples:** From a single collected sample, two or more subsamples are taken and stored (includes variability due to subsampling procedures and heterogeneity within a single collected sample).

3) **Collection of two series of sub-samples each of a different weight** (size). The results of analyses from each series of weight can be used to calculate Visman sampling constants for homogeneity and analyte segregation within the sediment and the expected subsampling variance (Ingamells, 1974).

4) **Field Control Samples:** Uncontaminated sediments are spiked with known amounts of the analyte(s) of interest.

5) **Preservative Reagent Blanks:** If a preservative is used, its quality should be routinely checked - see section 10.

6) **Control Site Selection:** When an objective of the program is identification of elevated levels of a particular analyte (contamination), a site or sites are usually selected that are thought to be uncontaminated for comparative purposes. Proper selection of control sites is important if data are to be correctly interpreted (sediment characteristics need to be closely matched). This is part of site selection (section 3.1.2).

7) **Selection of Proper Samplers:** Samplers must be selected which can retrieve the desired type of sediment, are non-contaminating and can collect a minimally disturbed sample. (See section 3.2)

8) **Selection of proper storage containers and storage conditions.**

Field control samples and replicate sample results must be treated with some caution. In field control samples, the added analyte may behave and be extracted differently than naturally occurring analyte. Quantitative recovery of the field spike does not mean naturally occurring analyte will be quantitatively recovered or behave in the same way. Under recovery however, will indicate a problem. Results for replicate samples will include variability due to the sampler, sample handling, storage, as well as the heterogeneity of the substrate. In some areas, the latter variability may be much greater than all other factors. Only replicate samples taken from a known homogeneous sediment can be used to estimate sampler and sampling technique uncertainty (precision).

3.5 Requirements for Legal Samples

Whenever it is known or suspected that samples will be required for litigation, additional precautions are required in sampling, handling, storage, transport and analytical procedures.

Dick and Pant (1978) summarized three fundamental objectives when dealing with legal samples for oil spills which can also be applied to sediment. These are:

1) the preservation of sample integrity so that the sample composition is the same as at the time of sampling;

2) the proof of continuity of the sample from sampling through to final documentation (chain of custody); and

3) the use of analytical techniques capable of providing positive identification of the pollutant.

Maintaining sample integrity is a function of the sampling and storage procedures and is the goal of all sampling programs. Available analytical techniques are discussed in Sections 6-10. The proof of continuity of the sample is to assure and document that the sample(s) is in the physical possession of a specified individual or in a secured place and could not have been damaged or altered (Katz and Jenniss, 1983). This requires special procedures not usually followed in routine applications. There are apparently no well defined rules to ensure continuity. Dick and Pant (1978) in their review outlined various methods in current use in Canada and the United States. The recommendations regarding sampling and sample handling are summarized below:

3.5.1 Sampling and Sample Handling

In preparation for field sampling, a sufficient number of appropriate containers should be assembled, with etched or permanently marked identifying numbers on the containers as well as on matching lids. The containers should then be properly cleaned and prepared, as specified for the particular analyte, by one lab person only. To reduce the possibility of contamination, the sample containers, lids and labels should be sealed individually into polyethylene bags. The containers are then packed into a locking sample box in readiness for field sampling. The box is marked "legal sample bottles", accompanied by the date and a signature of the person who prepared them, and then locked. At this point, the sample box key is given to the person in charge of sampling and the sample box is transferred by this person to the sampling site.

At the sampling site, field personnel are required to keep a field notebook of all pertinent information. For each sample taken the container number, date sample taken, time sample taken, source of sample (with reference to an area diagram), preservative, if used, and physical characteristics, where applicable, should be recorded in the field notebook as well as on the label supplied with each sample container. The field sampler's name and signature, as well as that of an emergency or investigating officer or witness should be recorded on each sample label (if possible). The label should then be securely fastened to the sample container and taped over with transparent tape to preserve the information. The container number is cross checked in the field notebook with the information on the label.

After being filled with the sample, each container is sealed and should be cross-taped and marked in such a manner as to prevent contamination and leakage, and to indicate if the container has been tampered with.

At least two sample containers should be left empty as a control, to prove that the containers were not contaminated before sampling. All possible suspected sources relative to each sampling site should be sampled, with a minimum of two samples from each location.

The sample box, with all the sample containers, is transported with the crew to each sampling location. Samples and the sample box itself, are not left unattended. After completion of sampling, the sample box is locked. The key should be kept on the field investigator's person at all times, and the sample box is clearly marked as to its contents. Samples must be in the field investigator's actual physical possession; or in an area where they can be kept under surveillance by an authorized person; or in a limited-key-access locked refrigerator or storage area, where they cannot be tampered with until such time that they can be brought to or sent to the appropriate laboratory for analysis.

Samples can be sent to the lab for analysis by registered mail, air freight or air express. Any transport documents associated with shipping, such as a hazardous material shipping form, are retained for the legal file. All wrappings and sample containing boxes are all similarly identified. A covering letter is prepared with similar identification and one copy is sent separately to the lab involved while another copy accompanies the samples. A memorandum describing the samples and the type of analysis required is included in the sample box. A key to the sample box is sent in a separate envelope and not attached to the box. The box lock is secured and taped-over. An added precaution that has been suggested is a photograph showing the condition of the box.

Hand delivery of the samples to the laboratory by the field personnel is preferred. The associated identifying documentation, memorandum of sample description and required analysis is also hand delivered. The laboratory person receiving the delivery and the person making the delivery sign a transmittal slip with copies being kept by both parties. The name of the person making the delivery is noted in the case file. A note of the transmittal is recorded in the laboratory record book or file and any additional receiving data is then noted, such as evidence of tampering. All packaging and wrappings are dated and initialled by the analyst and then retained on file.

Deliveries by registered mail or courier should be received by the analyst assigned the task of carrying out the analyses for the samples in question. Upon receiving the sample box, the analyst should take a photograph of it, to compare with a photo taken in the field. All registration slips or waybills should be kept with the box, and any wrappings initialled and retained.

With the arrival of the samples in the lab, a case file should be opened and case numbers assigned to each sample. A continuity sheet is prepared and the samples examined in conjunction with the memorandum requesting analysis.

All samples are marked by the analyst with his/her initials, the case number, the sample number and the date of receipt. All memoranda and associated documents are similarly marked, and the condition of the samples noted in the laboratory notebook.

One analyst should carry out all of the analyses and keep all samples, process solutions, etc., under lock and limited access key when not under direct surveillance. Notes are taken of all aspects of the analysis. All such notes, graphs, charts, and rough drafts of final reports should also be kept under lock and limited access key during the analytical process and when not in direct use.

Subsequent to laboratory analysis, the analyst should ensure retention of sufficient post-analysis sample for defence or rechecking purposes. Where adequate storage facilities exist, the post-analysis samples are retained under lock and limited-key-access until prosecution is completed or until the case is dropped.

If adequate locking storage space is not available at the lab or cannot be arranged, the analyst should return the samples with the final report to the originating field group. The samples are transferred either by hand, registered mail, air express, air freight or courier. If transferred by hand, a signature is obtained on the laboratory continuity sheet from the person receiving the shipment. If shipped by registered mail, express, etc., all documents involved in making the shipment and transmittal are retained in the case file. The receiver of the shipment signs the continuity sheet and holds the samples in a locked, limited access key area until the legal process is completed or the case dropped.

4. SAMPLING LOGISTICS

Implementing a sampling plan in the Arctic requires considerable logistic support and expense. Practical considerations must be taken into account and these will influence choice of samplers, sampling strategy and types of analyses. The factors to be considered in this section are:

- positioning
- transport
- sampling from the ice
- sampling from ships
- personnel requirements

4.1 Positioning

The required accuracy and precision of station positions will depend on the type of study and the size of the study area. Besides the performance capability, the other important considerations in the choice of a positioning method are:

- **range:** it is preferable to have one system that will cover the entire study area.

- **size:** the size and complexity of positioning equipment are important for transport.

- **cost:** this factor is obviously a major factor.

Recommended positioning systems are summarized for a variety of applications in Table 4.1. A brief description of other positioning systems is given in volume I, section II-6.

4.2 Transport

Sample transport from arctic regions is the portion of the analytical chain linking the field and laboratory portions of the study. This is a critical step, as the

TABLE 4.1

RECOMMENDED POSITIONING SYSTEMS FOR VARIOUS APPLICATIONS

SAMPLING PLATFORM	SURVEY TYPE	ACCURACY REQUIRED	APPROXIMATE COST[4]	SUGGESTED SYSTEMS
1. Shipboard	Wide area survey	100-500 m	$2,000 - 5,000	SATNAV[3](offshore) SATNAV[3] plus RADAR (nearshore)
		1-10 m	$50,000	ARGO, SYLEDIS
	Nearshore	10-100 m	>$5,000	RADAR[1]
		1-10 m		MINIRANGER/TRISPONDER MICROFIX
2. Small Launch	Nearshore	10-100 m	<$2,000	SEXTANT,PELORUS[1,2]
		1-10 m	$5,000+	MINIRANGER/MICROFIX/ TRISPONDER
3. Ice	Wide area survey	100-500m	$2,000 - $5,000	SATNAV
	Small area	10-100 m	<$2,000	SEXTANT, PELORUS THEODOLITE[1,2]
	Small area	1-10 m	>$5,000	MINIRANGER/MICROFIX/ TRISPONDER Lines along ice form fixed point.

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[1] suitable targets required

[2] Good visibility requird.

[3] Continuous positions ot available; dead reckoning required between fixe.

[4] Purchase prices most ystems can be leased or rented.

investigator loses control of the samples. Precautions must be taken to ensure the integrity of samples during transit and their timely delivery to the laboratory.

In the Arctic, all transport will ultimately be by air, perhaps with several transfers. The cost of air and ground transport can be a large component of the overall sampling expenditure. Charter fixed wing aircraft generally cost from $2.50 - $5.00/mile plus fuel. Helicopters cost from $500/hr plus fuel for a small 4 seater to over $1,000/hr plus fuel for larger machines. Prior to the trip, air carriers, routing, schedules and cargo restrictions must be known so that transport can be planned ahead. Few northern coastal communities have daily flights to southern Canada and all will have weight/size restrictions for cargo. A list of air carriers, licence restrictions and addresses for major arctic communities is given in Appendix C of volume 1. Some points to be considered when planning sample transport are:

1) cargo capacity of the aircraft (limiting dimensions for a single item and weight restrictions);

2) schedules: general availability of space, rates, priority freight service;

3) terminal facilities: are there facilities to keep samples cool, frozen, or to keep them from freezing in winter at transfer points?

4) restricted items: several items such as dry ice, concentrated acids, formalin, batteries (wet), gasoline cans, gasoline motors may not be accepted for carriage on passenger carrying aircraft. All restricted items require proper documentation regardless of aircraft and packaging. Failure to do this can cause delays or result in loss of necessary items (as well as potentially severe penalties). IATA (International Air Transport Association) publishes a listing of all restricted items, along with instructions for proper packaging, labelling and documentation. Permissable quantities are also indicated. The most recent IATA regulations should be consulted when planning a field trip (may be obtained from IATA, Traffic Services, 2000 Peel Street, Montreal, Quebec, H3A 2R4);

5) packaging: wooden, metal or hard plastic should be used when possible; some plastics become very brittle at low temperatures. All plastic containers should be tested before being used to transport materials in winter months. Cardboard boxes should not be used;

6) labelling: all items to be shipped need a number, the contents of each box should be recorded; labels should clearly show destination, special handling instructions and phone numbers to call on arrival; restricted items need proper documentation (item 4);

7) cost: charter rates, freight rates can be a major factor in the overall budget. In planning a sampling program, allowance should be made for the extra weight of samples on return trips;

8) timing: depending on the amount of equipment to be transported, it may be necessary to ship equipment ahead. When personnel follow, they can then ensure that all equipment reaches its destination on time.

The key to successful transport is good planning as well as the necessary flexibility to deal with forced delays due to weather.

4.3 Sampling from the Ice

Sampling from the ice is practical only when the ice is relatively stable. In nearshore areas this means when the ice has achieved sufficient thickness, usually a month or so after freeze-up until a few weeks (or less) prior to break-up. Further offshore and in many coastal regions multi-year ice may be present all year. Sampling from the ice has some advantages, most notably a stable platform and large surface area, as well as some obstacles not encountered in more traditional shipboard sampling, including access to the water, shelter and transport.

There are no standard procedures or equipment for sampling from the ice. Requirements and equipment vary with the application, transport restrictions, time of year, personal preferences and available resources. There are, however, several factors that should be considered when planning, namely:

1. site selection;
2. drilling holes in the ice;
3. positioning;
4. shelter and safety requirements;
5. transport;
6. equipment, power, winch and retrieval requirements; and
7. a practical sampling device.

The factors in sampler selection will be a function of goals, parameters to be analysed and portability. These topics have been reviewed in Sections 3.2 and 5. There is a practical limit to the size of hole that can be readily made in the ice. In general, the most compact sampler (smallest cross-sectional dimensions) capable of satisfying the objectives should be used. The other factors are discussed briefly below.

4.3.1 Site Selection

In first year, land fast ice, site location is usually not a problem. The ice is relatively flat and a uniform thickness. In transition zones or multi-year ice however, site selection is more difficult. This ice can be very uneven and broken. An area of flat first year ice is preferred as this will have a minimum thickness and provide a flat landing area for aircraft and equipment. Where the ice is ridged or tilted, ice thickness may be much greater than anticipated. This will require more time to make a hole in the ice or perhaps make it impossible with the equipment available. Multi-year ice will be thicker (2 - 10m) and harder in general. Sampling on transition or multi-year ice will require considerable flexibility in site location. The number of sites and their location will be dictated by local conditions and cannot be planned in detail in advance (Section 3.1).

4.3.2 Making a Hole in the Ice.

Once a location has been selected, a hole of sufficient size for the sampling gear is required. Small corers without stabilizer fins may require a hole of 25 cm diameter or less. Small grab samplers such as the Ekman will require a hole at least 30 cm in diameter while larger samplers like the Van Veen (short armed version) will require a hole for deployment at least 80 cm in diameter. If a small diameter auger is available, it may be advantageous to drill a small diameter hole first to determine ice thickness. This can save a lot of time if the ice proves to be too thick for the equipment available.

There are a number of ways of making a hole in the ice, depending on the ice thickness, size of hole required and the equipment available. In early winter or when the ice is relatively thin (30 cm or less) a sharp ice chisel is the simplest means available. The most common method however is to drill a hole using commercially available ice augers and power drives. The augers can be driven by gasoline powered engines, by electric motors, by a hydraulic drive or by hand. It is generally not practical to use a hand auger for holes greater than about 10 - 15 cm in diameter, which is too small even for gravity corers. With a power unit, either gasoline driven or electric, up to 45 cm holes can be drilled, though larger sizes (> 25 cm) require additional horsepower in the drive unit and are usually heavy. These units can penetrate 2 m of ice in about 10-20 minutes. If a large track vehicle is available, a vehicle mounted drill can be used driven by vehicle power or hydraulics and larger diameter augers used (up to 60 cm).

Portable gasoline powered engine drives have the advantage of portability, wide spread commercial availability, and low cost (typically under $1,000). The units are heavy however, and can be difficult to start in very cold weather. Two people will be required to drill smaller diameter holes (to 25 cm), with an extra person required for larger diameter units in thick ice.

Electric drills are also readily available. However, they require a power source (generator) which may present problems. Large drills with up to 1 hp motors are required.

For large size holes, chain saws with long extensions have been used. This is a laborious, time consuming (usually several hours) and messy procedure which is not recommended for routine work. However, for a limited number of sampling sites this is an effective means of making a large hole. For diving this may be the only practical method for making a large enough hole.

Perhaps the best and certainly most versatile method available is the hot water drill. Hot water drills are not available commercially but can be built from readily available components and materials. Verrall and Baade (1982) developed a unit for drilling holes in ice shelves along Ellesmere Island. They were able to put a 23 cm diameter hole through 50 m of shelf ice at a rate of 11 m/hour.

The Frozen Sea Research Group (FSRG) at the Institute of Ocean Sciences, Pat Bay, B.C., have also developed a hot water drill for use in arctic applications. The FSRG unit works on the same principle except that they utilize an oil burner (3 USGPH fuel consumption) and have produced a helicopter and Twin-Otter fixed wing aircraft portable design. FSRG also utilizes a melting ring rather than a single jet outlet. Two perforated rings are used in tandem, one for the outlet of hot water and one for collecting cooled return water. This system allows a great deal of flexibility as rings of various diameters can be easily fabricated depending on the application. Very large holes can be made by drilling several holes side by side. Using a 91.5 cm diameter ring, the FSRG unit can penetrate 2 m of ice in about 35 minutes. The ice block that is produced can either be pushed down under the ice or removed. The ice hole produced by either method has the additional advantage of being free of slush and ice chips produced in other drilling methods.

The chief disadvantages of this method are the requirements for a generator to run the pump and heater and the lack of a commercially available system.

4.3.3 Shelter and Safety

Except for ideal conditions in late spring or early summer when air temperatures may be above freezing, some sort of shelter will be required while on the ice. The type of shelter required will depend on the length of time spent on location, weather conditions, transport limitations and sampling gear. The simplest shelter is a windbreak but usually a heated area is needed both for personnel and equipment. This may be the transport vehicle (all terrain vehicle, snow-trak, airplane or helicopter); a portable insulated shelter or tent (Parcoll, Hansen or Norseman tent); a trailer that is either helicopter portable or mounted on skis; or a snow hut/igloo. Depending on clearance requirements and size of ice hole and sampler, sampling may be carried out within the shelter.

Sampling on the ice demands that the sampling party must be totally self-sufficient. Equipment requirements should reflect this and allow for emergency situations (communication equipment, survival gear, food, fuel, first aid equipment). Sampling on the ice also requires that the sampling party be aware of the dangers of an encounter with polar bears should the party be on the ice for a long time. Some sort of watch should be maintained and a rifle of suitable power (as well as someone with the knowledge of how to use it) must be readily available. Information on polar bear deterence strategies may be obtained from the Government of the Northwest Territories, Department of Renewable Resources, Habitat Management in Yellowknife.

4.3.4 Transport

Depending on the distance to be travelled and the amount of equipment required, a number of possible means of getting to a location are available. For short distances close to a community or camp, a snow vehicle (Snowmobiles, Bombardier, ATV or Cat) with a sled for equipment is suitable. Snowmobiles and sleds can be rented in any northern community. As distances increase, all transport will eventually rely on aircraft, either helicopters or fixed wing. Helicopters are more versatile but also more expensive to charter and not as readily available. Choice will depend on the area to be sampled, amount of equipment and distance to be travelled. Fixed wing aircraft are required when distances are great (generally more than 100 km). Helicopters have advantages on shorter flights in terms of flexibility (landing), in site selection and in handling bulky items (these can be carried in a sling under the aircraft). People cannot be transported simultaneously when equipment is carried in a sling. Aircraft cannot be used when visibility is poor, as a result of weather conditions, or darkness, so that long delays may result at certain times of the year.

4.3.5 Power, Winch and Lifting Requirements

With the exception of the small Birge-Ekman dredge, almost all sampling will require a winch and davit (or boom) to lower and retrieve the sampler (see Table 4.2). Lifting requirements and the size of winch and power required as much as the sampler dimensions will dictate the type of sampler that can be used. All equipment must also be portable enough for aircraft transport. Winches can be gasoline powered, electric or hydraulic. Drive units for ice augers can often be adapted to drive a winch. Tripods or quadrapods have been used to support the block above the ice hole. Winches can be supported directly on the frame.

There is no commercially available equipment "ready to go". Equipment components must be designed and mated to satisfy the sampling needs. Experience from others who have sampled from the ice will be helpful. Ultimately, however, investigators must adopt methods to suit their specific requirements and must thoroughly test out equipment and procedures before finalizing a sampling plan.

4.4 Sampling From Vessels

Vessel requirements for sediment sampling in arctic waters will be the same as for other marine areas with the exception that, in some instances, vessels with an ice-strengthened hull will be required. **A major constraint to programs requiring a large scientific vessel is the general lack of suitable vessels in the Arctic.** Choices will be very limited and costs will be higher. Charter costs for a 40' fishing vessel with operator **when available** will be in the range of $2,000/day plus fuel. Larger vessels must usually be brought in from southern areas at great expense. In more remote areas, the only boats available may be 15' - 20' fishing boats or canoes, or inflatable boats flown in. This will obviously restrict the type of samplers that can be used and the sampling plan (section 3).

The following factors should be considered when formulating a sampling plan for sampling from a boat:

1) lifting capability: winches, line (strength, material and length), blocks, booms, cranes, A-Frames, davits; (see Section 2.3 for approximate lifting requirements; see U.S. Naval Oceanographic Office, 1968; Stirn, 1981, or Holme & McIntyre (1984) for details). Table 4.2 outlines typical applications for various types of winches and wire rope;

2) clearance over the railing with available lift capacity (function of sampler dimensions);

3) open work area for sampler manipulations, storage, etc.[a]
(this will depend on the type of sampler, the amount of equipment and number of people required to launch and recover sampling gear);

4) laboratory space (for sub-sampling, sample work-up);[a]

5) storage facility (freezer and refrigerated space);

6) navigation equipment (see Section 4.1);

7) accommodation;

8) range (fuel capacity, rate of consumption, speed);

9) anchoring capability;

10) availability of electrical power;

11) availability of running water (benthic sampling);

12) overall dimensions, sea-worthiness, ice rating; and

13) communications - types of radios, frequencies available and range.

[a] Note: Ships present very dirty environments for sample handling. Special care must be taken to prevent contamination.

TABLE 4.2

TYPICAL APPLICATIONS FOR VARIOUS WINCHES AND WIRE ROPE LINES

DUTY	APPLICATION	WIRE ROPE SIZE (mm)	LENGTH OF LINE (m)	LOAD (kg)	SPEED (m/min)	MOTOR (H.P.)
Light	smallest grabs	2.4 - 3.2	400 - 800	50	300	3 - 5
Medium	all grabs, light to medium weight cylindrical and box corers	4 - 5	up to 5000	250 - 500	100 - 300	15 - 20
Heavy	piston corers, large box corers and gravity corers	9.5 - 12.5	to 12,000	14,000 - 3,600	40 - 120	30 - 150

4.5 Personnel Requirements

An important part of any study plan is the allocation of personnel. Given the uncertainties in field programs, the necessity for compromise and adaptations, the quality of field personnel may be considered one of the most important factors in a successful sampling program. Both the number of people and their level of training and experience are important considerations. There are no set rules for estimating personnel requirements, although certain tasks require a minimum number of people regardless of training.

Table 4.3 lists guidelines for establishing personnel requirements for various types of sampling apparatus. However, if sample processing or work-up is required while sampling is taking place, then additional personnel will be required. At least one member of the sampling team must be experienced in all the techniques to be used.

Table 4.3

Personnel Requirements: Sampling Methods

Type of Sampler	Relative Difficulty	Number of People Required to Deploy and Retrieve
A. Grabs		
Small grabs (e.g. Ekman, Ponar)	simple; requires little training or technical skill: easy to handle because of small size as long as deployed in shallow water	1 (possibly 2)
large grabs (Van Veen)	simple; requires little training to successfully deploy; heavier	usually 2 (and winch)
grabs with frames (Smith-McIntyre)	most complicated of grabs to operate; heavier and more bulky; however, these grabs can be used with minimal training	2 (and winch)
B. Corers		
cylindrical gravity	easy to use; little training required	2 (and winch)
box corer	most complicated; requires an experienced person to operate successfully: training in field almost essential	2 - 4 (and winch)
piston	complicated, potentially dangerous: awkward to handle; should be handled by experienced personnel only	2 - 4 (and winch)
damped, framed (Craig)	complicated; requires previous training	2 (and winch)
C. Diving	must be experienced divers	minimum of 2

Note: The most demanding part of sampling is being able to recognize problems (i.e., disturbed sediment; excessive washing) and being able to adapt equipment to changing conditions. This cannot be taught - actual field experience is the most important factor. Although no specialized educational background is required some technical competence and a thorough understanding of the principals of how the sampler works are required.

Subsampling requires special care: the person subsampling must be familar with the study goals; sources of contamination and how this can be avoided. A trace metal or trace organic chemist is not necessarily required. However, very explicit instructions need to be provided if a chemist is not available. The instructions should be clearly written out by the study scientist in conjunction with the chemist responsible for the analyses.

Field processing (sieving) of sediment for benthos requires someone with a basic knowledge of benthic organisms. Explicit instructions need to be provided if a benthic biologist is not available to carry out preliminary processing.

5. METHODS FOR THE STUDY OF MARINE MACROBENTHOS

5.1 Introduction

The objective of this section is to outline the basic steps in planning and carrying out applied environmental studies on marine macrobenthos. The approach taken here is to first indicate major elements for planning applied environmental studies. These include (a) the conceptual steps in planning and executing a study and (b) basic considerations of sampling design and evaluation. Selection of suitable benthic sampling devices is included as a facet of sampling design. Next, the five major types of applied biological studies are described along with a framework for deciding which might be applicable to a particular sampling problem. For each main category, the basic options for sampling design, statistical analysis and sampling allocation are outlined.

Finally, the procedures for sampling macrobenthos and methods for treatment of benthos samples are considered.

Excellent general references on sampling design and statistical methods for applied biological studies are Elliott (1977), Green (1979), and Allan (1984). If the user is not familiar with statistical tests it is recommended that the advice of a statistician be obtained at the planning and design stage rather than after the samples are collected and analysed.

5.2 Elements of Planning for an Applied Study of Marine Benthos

The structure of an environmental study should be based on the logical progression from objective → question → hypothesis → sampling → design → statistical analysis → tests of hypotheses → interpretation and reporting of results (Figure 5.1). The biologically defined objective must preside over and use the statistical methods instead of the opposite (Skellam, 1969; Green, 1979).

5.2.1 Hypothesis Formation

The first step is to form hypotheses from the questions arising from the objective of the study. The framing of a testable and falsifiable null hypothesis (H_0) is necessary before a statistical test can be applied. This means that the results

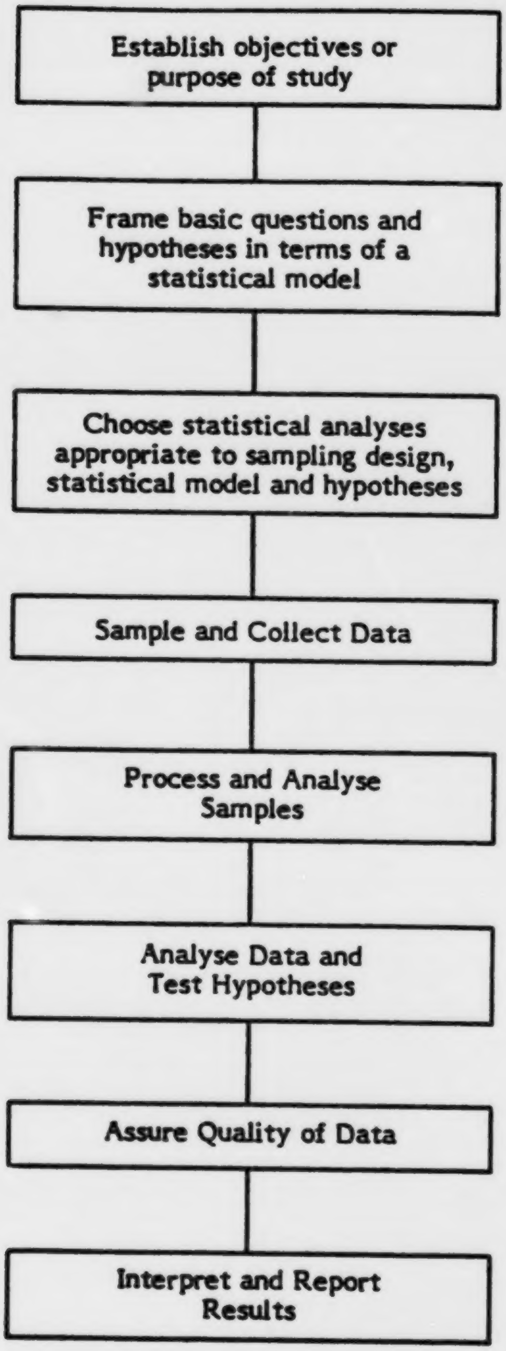

Establish objectives or purpose of study
Frame basic questions and hypotheses in terms of a statistical model
Choose statistical analyses appropriate to sampling design, statistical model and hypotheses
Sample and Collect Data
Process and Analyse Samples
Analyse Data and Test Hypotheses
Assure Quality of Data
Interpret and Report Results

Figure 5.1 Conceptual Steps in Planning and Executing an Environmental Study.

cannot be explained so that they support a given hypothesis regardless of what the results are. There must be possible results that require each of the two possible decisions: accept H_0 or reject H_0. The null hypothesis proposed should be the simplest possible answer to the question arising from the purpose of the study. Sampling and statistical analyses follow from this.

When statistical analysis is applied for exploratory descriptive purposes, tests of H_0: "nothing is going on" may be appropriate. For example, multivariate methods such as cluster analysis and ordination are often applied to reduce large, complex data sets to "what is going on". Because these procedures can give results that have the appearance of something going on even when used on random simulated data, it is advised that the H_0: "nothing is going on" be rejected before proceeding with this type of descriptive analysis (Williams and Lance, 1965; Goodall, 1966; Green, 1979).

5.2.2 Statistical Models

A proper null hypothesis is generally expressed in terms of a statistical model which states some hypothesized functional relationship between one or more dependent variables and one or more independent variables. For example, the dependent or criterion variables could be measures of biological impact effects. The independent or predictor variables could be measures of impact intensity or also of environmental variation which may contribute to variation in the criterion variables even though it is not related to the impact itself. The perfect statistical model would have the properties of generality, realism and precision (Levins, 1966). In practice, one of these must be jeopardised, which determines the strategy to be taken. For instance, generality may be lost if one is concerned with predicting what will happen at a specific location. In another case, a high degree of precision may not be needed if a biological response has a large amplitude. It is important, to give considerable thought to the choice of an appropriate statistical model because this can be a more serious source of error and deception than errors in estimation or hypothesis testing (Skellam, 1969; Green, 1979).

5.2.3 Sampling Design and Evaluation

The sampling design should be as compatible as possible with the statistical model of the null hypothesis (Steel and Torrie, 1960; Elliott, 1977; Green, 1979).

Criterion and predictor variables must be chosen carefully to ensure they really measure what they are intended to. Therefore, the choice of an appropriate sampling device and an understanding of its limitations in sampling the target population are important facets of sampling design (Figure 5.2). Factors to consider in the choice of a suitable sampling device for benthos are:

1) nature of the substrate (hard or soft)
2) target population (infauna or epibenthos)
3) desirability of direct observation of benthos and habitat
4) depth of study area (<20 m or deeper)
5) sampling platform (vessel in open water, or from ice)
6) cost

A flowchart of decisions and possible solutions is given in Figure 5.3. Characteristics, advantages, disadvantages and approximate costs of various types of sampling equipment for macrobenthos are provided in Table 5.1. General references on sampling gear for benthos are Holme and McIntyre (1971, 1984) and Holme (1964). A review provided in Sections 4 and 5 of Volume I considers various types of remote and direct samplers for macrobenthos.

A highly desirable feature of sampling design is that sampling be truly random at some level. If not, the assumption of independence of errors may be violated, making most statistical tests invalid. This condition is impossible to remedy after the data have been collected, but can be avoided by truly random allocation of samples. Further discussion of this topic is presented later in relation to error variation.

Sampling design should aim to control or hold constant as many as possible of the environmental variables which may be correlated with the biological criterion variable(s). For example, in the design of an impact study, control or reference areas and impact areas should be selected so that they have similar environmental conditions except for the projected presence or absence of the future impact.

Equal numbers of randomly allocated replicate samples should be taken within each combination of location, time, and any other controlled variable. For most statistical tests of hypotheses, an estimate of the amount of variation within as well as variation among each combination is needed. Estimation of variation within requires replication. A good example of an environmental study with consistent,

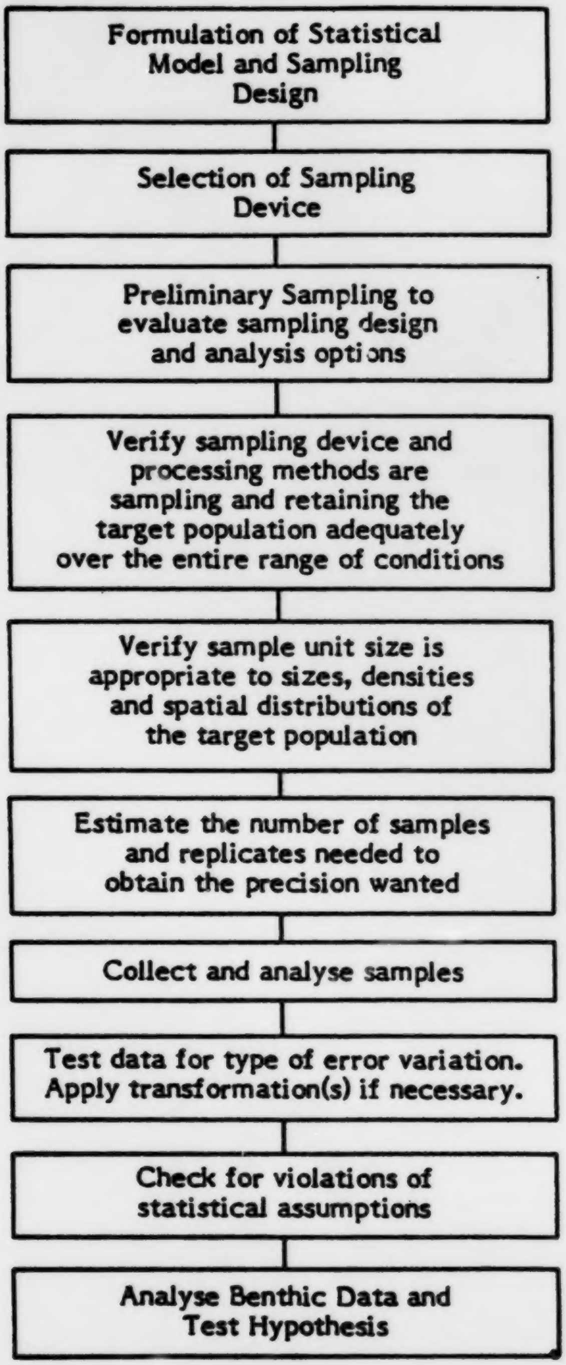

Formulation of Statistical Model and Sampling Design
Selection of Sampling Device
Preliminary Sampling to evaluate sampling design and analysis options
Verify sampling device and processing methods are sampling and retaining the target population adequately over the entire range of conditions
Verify sample unit size is appropriate to sizes, densities and spatial distributions of the target population
Estimate the number of samples and replicates needed to obtain the precision wanted
Collect and analyse samples
Test data for type of error variation. Apply transformation(s) if necessary.
Check for violations of statistical assumptions
Analyse Benthic Data and Test Hypothesis

Figure 5.2. Sampling Design and Evaluation

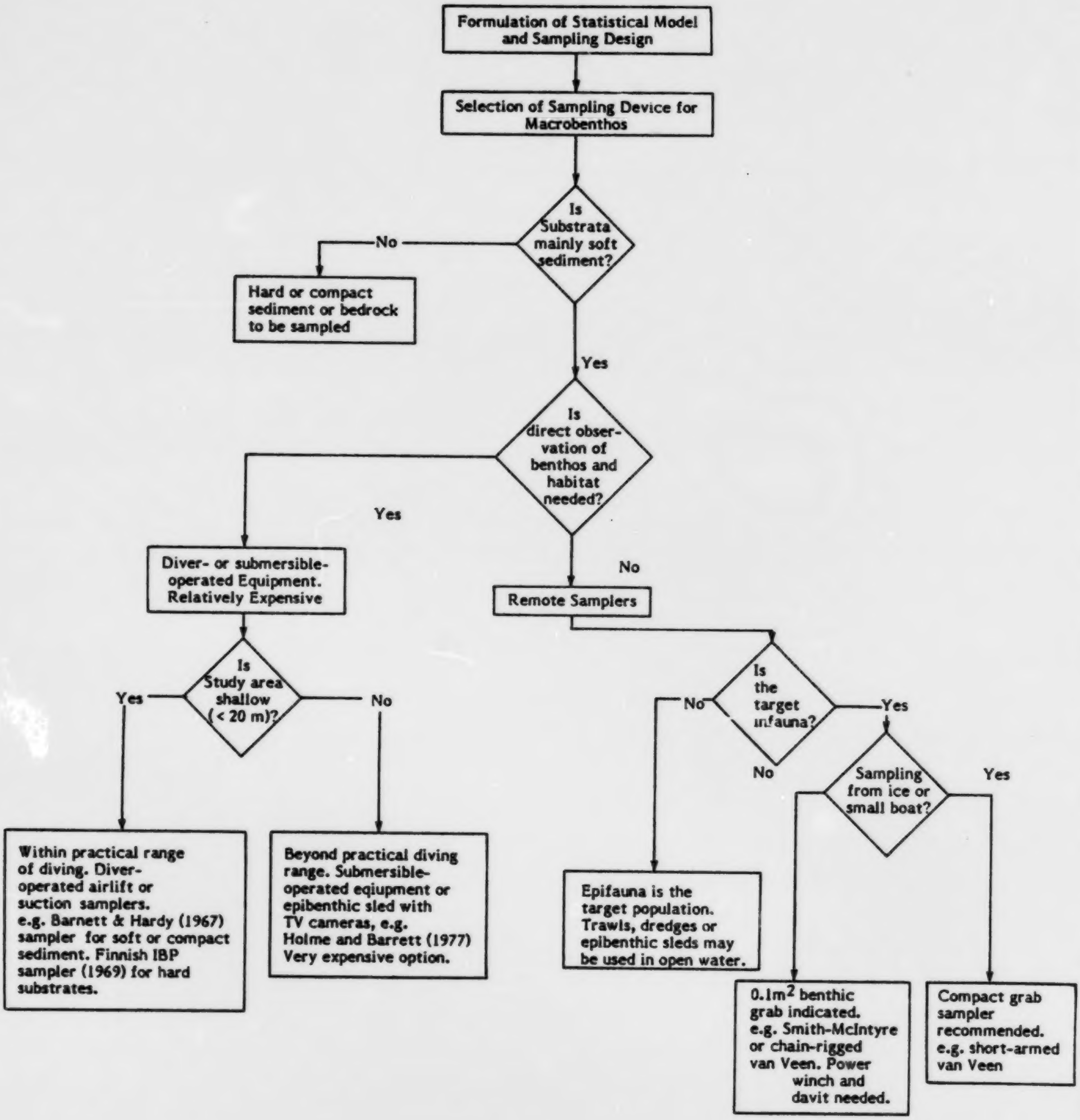

Formulation of Statistical Model and Sampling Design
Selection of Sampling Device for Macrobenthos
Is Substrata mainly soft sediment?
No
Hard or compact sediment or bedrock to be sampled
Yes
Is direct observation of benthos and habitat needed?
Yes
Diver- or submersible-operated Equipment. Relatively Expensive
No
Remote Samplers
Is Study area shallow (< 20 m)?
Yes
No
Within practical range of diving. Diver-operated airlift or suction samplers. e.g. Barnett & Hardy (1967) sampler for soft or compact sediment. Finnish IBP sampler (1969) for hard substrates.
Beyond practical diving range. Submersible-operated eqiupment or epibenthic sled with TV cameras, e.g. Holme and Barrett (1977) Very expensive option.
Is the target infauna?
No
Yes
No
Epifauna is the target population. Trawls, dredges or epibenthic sleds may be used in open water.
Sampling from ice or small boat?
Yes
0.1m² benthic grab indicated. e.g. Smith-McIntyre or chain-rigged van Veen. Power winch and davit needed.
Compact grab sampler recommended. e.g. short-armed van Veen

Figure 5.3 Selection of a Suitable Sampling Device for Macrobenthos Sampling.

Table 5.1

Summary of Methods of Macrobenthos Sampling

Sampling Device	Type of Information	Sampling Habitat	Organisms or Parameters	Typical Sampling Area	Unit of Measure	Limitations of Use	Approximate Cost	References
1. Grab	Quantitative	Soft or fine sediments	Infauna; biomass, density	0.1 or $0.2m^2$	$g\text{-}m^{-2}$ $no.\text{-}m^{-2}$	Soft sediment, limited area	van Veen $1500 Smith-McIntyre $5,000	Petersen 1977 Carey & Ruff 1977
2. Airlift	Quantitative with quadrat or cylinder	Hard or soft substrates	Epibenthos and infauna; biomass, density	0.05 to $1m^2$	$g\text{-}m^{-2}$ $no.\text{-}m^{-2}$	Shallow depths (<20m), and good visibility only; diver-operated	To fabricate $500 $1000	Barnett & Hardy 1967 Finnish IBP-PM Group 1969
3. Trawl	Qualitative (or quantitative with odometer)	Hard or soft even substrate	Epifauna, (biomass, density with odometer)	Unknown (or estimated from trawl odometer)	Comparative (or $g\text{-}m^{-2}$ $no.\text{-}m^{-2}$)	Open water, free of obstructions; smooth bottom	Beam trawl $2000-$3000	Carey & Ruff 1977 Carney & Carey 1980
4. Dredge	Qualitative or semi-quantitative	Hard or soft substrate	Epibenthos	Unknown	Comparative	Open water, clear of ice	Rectangular dredge $1000	Mohr et al 1957 Lee 1973
5. Epibenthic sled	Qualitative or quantitative with 6. and 7.	Hard or soft substrate	Epibenthos; density, biomass	Estimated from tow duration	$no.\text{-}m^{-2}$ $g\text{-}m^{-2}$	Open water, free of obstructions	Sled $1000-$2000	Holme & Barrett 1977
6. Still camera	Qualitative or quantitative with 3 or 5.	Hard or soft substrate	Epifauna; density	Various (e.g. $0.5 - 1m^2$)	$no.\text{-}m^{-2}$	Good visibility only; only with other devices (3,5)	Diver camera $1000-$3000 Remote camera $3000-$5000	Johnston et al 1969 Cross & Thomson 1981 Dyer et al 1982
7. Television	Qualitative or quantitative with 3 or 5.	Hard or soft bottom	Epifauna, density	Various	$no.\text{-}m^{-2}$	Good visibility only; only with other devices (3,5)	Black/White $1000-$10000 Colour $17000-$20000	Holme & Barrett 1977

randomly allocated sampling for each combination of location and time is presented by DeMarch (1976). Sokal and Rohlf (1973) have discussed and illustrated the principle of replication in relation to the concept of the F-statistic and its multivariate analogues. Pooling or combining of replicate samples eliminates the basis for estimating variation within and therefore should not be done except under special circumstances (see Brown and Fisher, 1972; Rohde, 1976).

If the study area has a large-scale pattern of environmental conditions, stratified sampling may be appropriate. If density variation is of principal concern, the area can be divided into relatively homogeneous subareas or strata within which the samples are randomly allocated in proportion to the size of the stratum. For example, if the study area has soft muddy sediments interspersed with gravel bars which account for one-fifth of the surface area of the local sea bottom, then the two subareas of widely different sediment types can be treated as strata with sample representation in the ratio 4 mud: 1 gravel in the sampling design. The variation in benthos abundance (or other criterion variables) between the strata can then be removed from the analysis, resulting in only the variation within strata entering the error variation term of the appropriate statistical test. Steel and Torrie (1960) and Cochran (1963, 1977) have described examples of stratified sampling with an estimation of the gain in efficiency of the statistical analysis from the use of a stratified as compared with a completely random design.

5.2.4 Preliminary Sampling and Background Data Acquisition

As indicated above, selection of an appropriate sampling design for a field study requires certain preliminary or background information on environmental conditions. In general, the more information available on an area before designing a sampling program, the better the sampling and its results. For proper planning of quantitative benthic studies, the following information is needed:

(1) Bathymetric data for the study area from a nautical chart or echo sounding surveys (summarized on a map of the area).

(2) Sediment data from all available sources (e.g. nautical charts, geological reports etc.). This information, in addition to data on the distributions of distinct water masses within the area, is one of the most important elements in planning quantitative benthic sampling (see Section 5.2.3).

(3) Oceanographic data on distribution of water masses and their movements, and tropic conditions in the pelagic environment of the study area. These data are necessary for planning and for data interpretation.

(4) Baseline information on chemical parameters if pollution studies are to be undertaken. This is necessary for selection of control or reference areas and delineation of the study area.

(5) Qualitative data on the types of benthic communities and their composition.

All of the existing information should be compiled and updated or extended by preliminary sampling fo the benthos and substrate. Preliminary sampling is especially useful for identifying potential sampling problems and for evaluating the sampling methods and statistical analysis options. On the basis of preliminary sampling, the number of samples needed to obtain a desired level of confidence can be estimated. This topic is covered in Section II - 3.1.3, Volume I and in general references such as Cochran (1977), Elliott (1977) or Green (1979).

Preliminary results may also be used to test for the type of error variation. Decisions in the use of transformations or nonparametric tests can be made on this basis. An examination of preliminary data for possible violation of assumptions of statistical methods can greatly aid in the choice of the appropriate statistical options. Basic references on the assumptions of classical statistical methods and the consequences of their violation are Eisenhart (1947) and Cochran (1947). The subject has been reviewed by Glass et al. (1972), with emphasis on the consequences of specified violations in simulated data. Their conclusions on the robustness of many statistical methods to violations of assumptions are reassuring. They conclude that correlated errors can have more serious consequences on the validity of statistical tests than all other violations. (Random sampling, at least at some level of sampling design, will prevent that violation.) If the errors are positively correlated, the test will tend to be more liberal than the stated level, whereas it will be more conservative if the errors are negatively correlated (Glass et al., 1972).

5.2.5 Statistical Analysis

The statistical model should be determined by the choice of the hypothesis model. The analysis used depends, therefore, on the information required to test the hypotheses and on the sampling design. For this reason, it is critical that the purpose of the study and the expected methods of analysis are established in the planning stage rather than after all of the samples are collected.

In addition to being compatible with the hypothesis model, the chosen type of statistical analysis should be efficient, in other words, be as conservative, powerful and robust as possible. Being conservative implies having a low probability α of making the Type I error of incorrectly rejecting H_o. Being powerful means having a low probability β of making the Type II error of accepting H_o when it is false. Being robust signifies that the stated error levels will not be seriously affected by the kinds of data commonly collected in environmental studies, which rarely satisfy all of the assumptions of statistical models (cf. Section 5.2.3). Robustness of statistical inference will be increased by the use of several analysis methods based on different assumptions. If the methods, despite their different assumptions, lead to similar results, then the decisions will be robust or relatively free of the assumptions of the methods. This approach can be particularly useful where performing exploratory analyses on multivariate data sets where hypotheses are poorly defined (e.g. Section 5.2.1) (Green, 1979). For example, a null hypothesis that there are no natural groups or clusters in the samples might suggest the use of cluster analysis or ordination procedures. If several clustering or ordination techniques, based on independent algorithms, all produce the same grouping of samples, then the decision that there are real groups of some kind is a robust one.

5.3 Classification of Applied Biological Studies

Applied environmental studies generally can be divided into three major classes: baseline, monitoring and impact studies. Impact studies have several variations as well (Section 5.3.1). These terms have been used loosely in the literature, but the definitions given below, after Green (1979), are used in this guide.

(a) Baseline Studies

A baseline study has a descriptive goal (Section 3.1) and is done to define or describe the present ("baseline") state of the biological community, the environment, or both. Generally some environmental change is projected, but the nature of the change and its timing may be unknown. If spatial and temporal (e.g. seasonal) variation of biotic and environmental variables occur in the system, they should be represented in the baseline model (e.g. Section 5.3.1.2).

(b) Impact Studies

An impact study has the objective of determining whether a specified impact affects a biological community, and if so, to describe the nature of the change. It may be possible to collect both before- and after-impact data (permitting an optimal impact study design) or only after-impact data. There may or may not be a control or reference area which does not receive the impact. **The nature of the impact and the certainty of its occurrence is always known.**

(c) Monitoring Studies

A monitoring study has the objective of sampling to detect a change from the present state. If detection of a change in a biological community is involved, then it is a biological monitoring study. Baseline data must have been collected to provide a standard against which a change might be detected. To permit the highest sensitivity of monitoring studies in detecting effects, the change should be clearly defined by using the results from earlier impact studies on the same or similar system. This definition provides a specific alternate hypothesis against which to test the null hypothesis of no detectable change.

5.3.1 Impact Study Designs

An impact study is best designed when it contrasts impact effects to previously collected baseline data. It's highest or optimal level of use is achieved when the results provide the basis for subsequent monitoring to detect future impacts of the same type.

An optimal impact study design has four requirements:

1. The impact must not have yet occurred; before-impact baseline data thus establishes a temporal control against which the after-impact data can be judged.

2. The type of impact and its timing and location must be known so that a sampling design suitable for tests of hypotheses can be devised. This property distinguishes it from a monitoring study to detect change.

3. Measurements of all relevant biological and environmental variables must be obtainable in association with the individual samples to permit hypothesis testing.

4. A non-impacted area must be available to serve as a spatial control or reference area.

Reference standards in both time and space are necessary for an optimal impact study design because evidence for impact effects on the biological community must be based upon changes detected in the impact area that were not observed in the reference area. Impact studies which lack one or more of the above requirements are "suboptimal."

A flow chart (Figure 5.4) conveniently illustrates the distinctions between five categories of applied biological studies. The distinctions are based on three decisions regarding design prerequisites. The questions are (a) whether the impact has already occurred; (b) when and where it occurred; and (c) whether a control area is available. From the responses it is possible to decide which category of study design is appropriate in a given situation. In this scheme, baseline and monitoring studies are combined in one category since the specific time and place of range from the present state are unknown. Notice that categories 2 and 4 are suboptimal impact studies which lack a spatial or a temporal control, respectively. Category 5 is the worst case, lacking controls and information on origins or the impact. The last category is rather uncommon and requires specialized strategies. Principal emphasis is on the first four categories. Within each of the five study categories, the general planning steps discussed in Section 5.2 are applicable. More specific recommendations for each study category are given in following sections.

5.3.1.1 Optimal Impact Study Design and Analysis

If an optimal impact study design is indicated by the decisions in Figure 5.4, then the options for sampling design and statistical analysis will be similar to those outlined in Figure 5.5. Due to its requirements for sampling multiple areas and times, an optimal impact study design is necessarily an area-by-times factorial design in which the evidence for impact effects is a significant areas-by-times interaction.

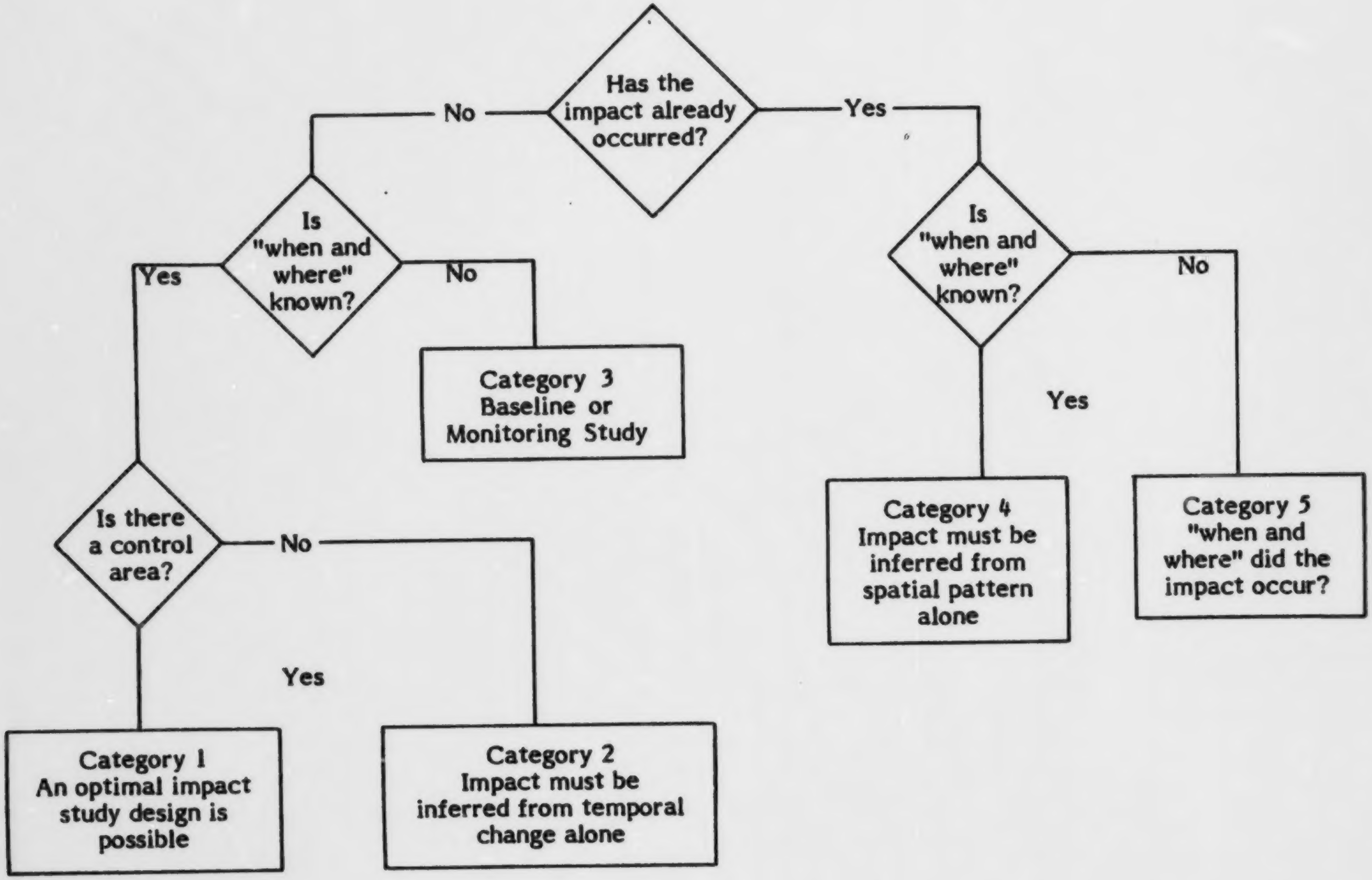

Has the impact already occurred?
No
Yes
Is "when and where" known?
Yes
No
Category 3 Baseline or Monitoring Study
Is there a control area?
No
Yes
Category 1 An optimal impact study design is possible
Category 2 Impact must be inferred from temporal change alone
Is "when and where" known?
No
Yes
Category 4 Impact must be inferred from spatial pattern alone
Category 5 "when and where" did the impact occur?

Figure 5.4 Flowchart for decisions on the major categories of applied biological studies (after Green , 1979).

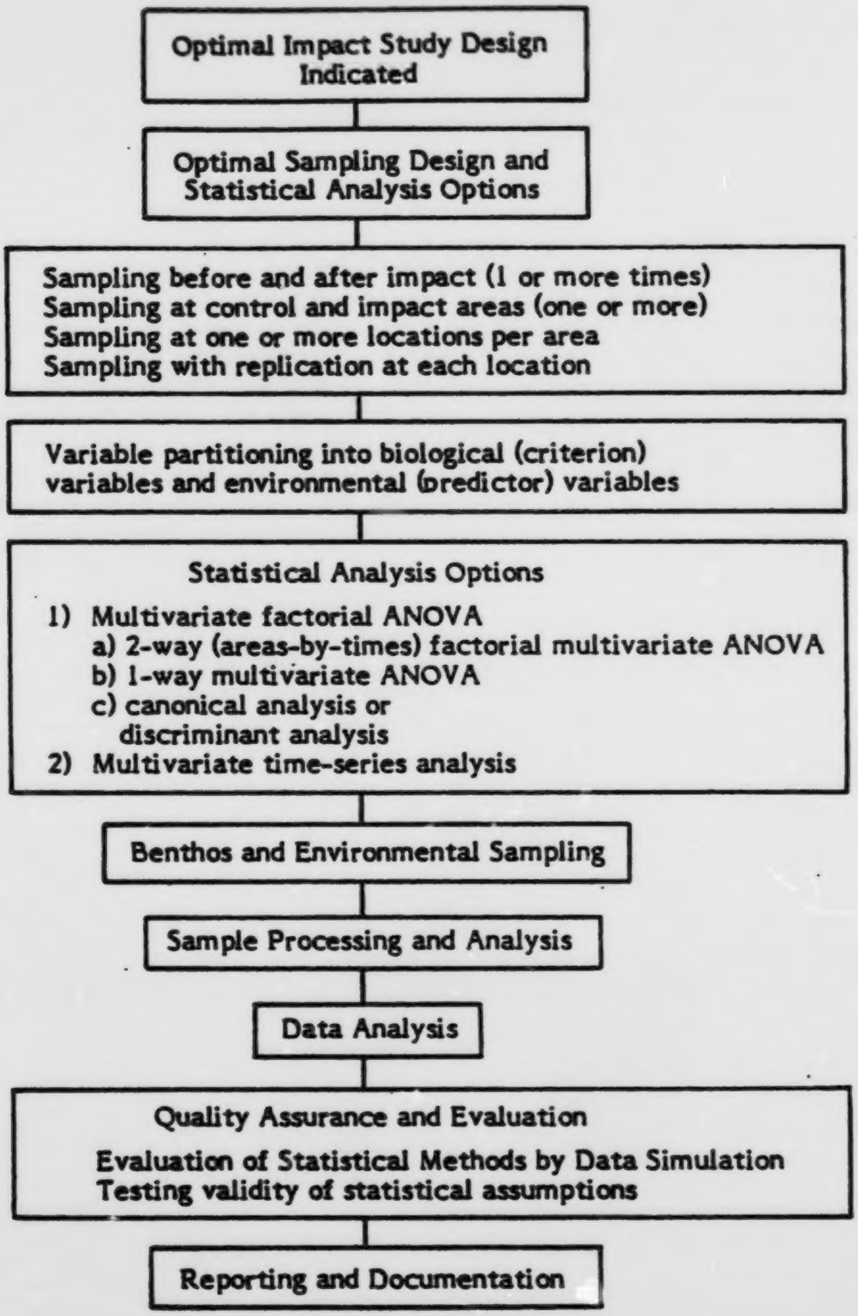

Optimal Impact Study Design Indicated
Optimal Sampling Design and Statistical Analysis Options
Sampling before and after impact (1 or more times)
Sampling at control and impact areas (one or more)
Sampling at one or more locations per area
Sampling with replication at each location
Variable partitioning into biological (criterion) variables and environmental (predictor) variables
Statistical Analysis Options
1) Multivariate factorial ANOVA
a) 2-way (areas-by-times) factorial multivariate ANOVA
b) 1-way multivariate ANOVA
c) canonical analysis or discriminant analysis
2) Multivariate time-series analysis
Benthos and Environmental Sampling
Sample Processing and Analysis
Data Analysis
Quality Assurance and Evaluation
Evaluation of Statistical Methods by Data Simulation
Testing validity of statistical assumptions
Reporting and Documentation

Figure 5.5. Category 1: Optimal Impact Study Design

As suggested by the ANOVA terms used, ANOVA is a highly appropriate method for statistical analysis of an optimal impact study. This method and the other options in Figure 5.5 will be discussed later in this section.

The final choice of a particular sampling design and statistical procedure should be based on the following criteria:

1. There must be a testable null hypothesis that any change in the biological community of the impact area, over a time interval which includes the impact, does not differ from a control or reference area.
2. One should be able to relate to the impact any significant change unique to the impact area and to separate effects caused by natural environmental variation not connected with the impact.
3. The method of data analysis should be conducive to an effective visual representation of both the effects due to impact in relation to other sources of variation and the relationship between impact-related change in the biological (criterion) variables and input-related change in the predictor variables.
4. The results of the analysis should be suitable for later use in biological monitoring to detect future impacts of the same type.
5. The test of the null hypothesis that no change due to impact occurred should be as conservative, powerful and robust as possible (see Section 5.2.4).

Multivariate factorial ANOVA methods are often well suited for application to the optimal impact study situation (Figure 5.5). In the two-way (areas-by-times) factorial multivariate ANOVA design, the test of the null hypothesis of no impact effects in the biological community corresponds to the test of the null hypothesis of no area-by-times interaction for the biological variables. The equivalent null hypothesis may be tested for impact effects on environmental conditions by using the environmental variables. Samples may be allocated within the four areas-by-times combinations in either of the following ways: (a) randomly or (b) randomly within each of a number of randomly positioned locations nested within the areas-by-times combinations. An example of (b) for sampling of benthos might involve random nested sampling by grab (e.g. randomly allocated replicate sampling) at randomly positioned locations within the impacted and non-impacted areas at times before and after the impact occurs. Sampling design (b) permits accounting for among-location-within-area variability caused by a heterogeneous environment. A further example of nesting might involve random subsampling of the grab samples with small corers (e.g. Hamilton et al., 1970). Examples of applications of higher order multivariate ANOVA designs are presented by Mager (1974) and Harris (1975).

If the sampling locations are randomly allocated in each area for the initial before-impact sampling, and then the same locations are sampled each subsequent time, then the data will be in a form suitable for analysis by one-way multivariate ANOVA. (Note that in the factorial design, sample locations are randomly allocated in each of the four areas-by-times combinations.) An example of a one-way multivariate ANOVA design to detect impact effects on a benthic species assemblage is to sample randomly allocated locations in the impact and control areas to obtain two groups of data on changes in species abundance variables (e.g. percentage change; Green, 1979). A one-way multivariate ANOVA would test the null hypothesis that the mean values of the species change variables for members of the species assemblage do not differ between the two areas. This design is similar to that used in a paired t-test. Green (1977, 1979) has presented an example for benthic communities based on this design, but lacking a control area.

Advantages of the one-way design over the factorial design include:

(a) simpler sampling and analysis design.

(b) computer programs for statistical analysis are more widely available for one-way multivariate ANOVA.

(c) alternatives are available for one-way ANOVA for remedying violations of statistical assumptions (e.g. homogeneity of within-area variance-covariance matrices), such as nonparametric procedures (Kendall, 1966; Mantel, 1970). Nonparametric analogues have not been developed for factorial designs.

(d) the possibility of regressing the species change variables or environmental change variables exist for one-way designs but not for factorial designs.

On the other hand, the disadvantages of the one-way analytical design in relation to the factorial design include:

(a) in the one-way design, the random sample is equivalent to the random location, which is fixed for pre- and post-impact sampling times. The relative influence of an odd or atypical location may be increased since it will be sampled twice during the study.

(b) conversion of species abundances to differences will tend to increase the sampling error because the variance of a difference equals the sum of the variances of the raw values.

(c) the one-way analytical design does not correspond exactly to the factorial sampling design. The factorial ANOVA design, however, has a direct and logical correspondence with the optimal impact study design.

Multivariate ANOVA and related subjects such as canonical analysis and discriminant analysis have been reviewed by Cooley and Lohnes (1962, 1971), Marriott (1974) and Harris (1975). Green (1979) has presented a worked example of canonical analysis, which is closely allied to multivariate ANOVA, in an optimal impact study on simulated benthic data. An advantage of canonical analysis is that critical values of the canonical variates can be used as possible monitoring criteria for detecting future impacts in the system. This technique will be discussed in more detail in Section 5.3.1.3.

Another approach to optimal impact study design is that of multivariate time-series analysis, a term which covers a broad group of techniques. Basic references on these methods are by Anderson (1963, 1971), Munn (1970) and Batschelet (1976). This methodology requires a series of observations over time, adequate to demonstrate the nature of the pre-impact temporal pattern of the studied system. After a specified time when impact occurs, the null hypothesis of no change in this temporal pattern may be tested. Optimal sampling design requires that a control or reference area be sampled and analyzed in a parallel fashion. For environments with marked seasonal variation and with impacts occurring over extended periods and areas, the approach of time-series analysis has considerable merit. The major drawback is that such studies need specialized temporal sampling designs with a large number of samples, and consequently a large operating budget, especially if the sampling is done in remote areas. Ideally, the ANOVA sampling design and analysis based on two sampling times circumvents rather than follows any natural temporal variation by isolating the impact in time through sampling done just before and soon after the event. In cases where the impact occurs over an extended period of time, the results of the multivariate ANOVA can be included in a monitoring study (Section 5.3.1.3).

5.3.1.2 Suboptimal Impact Study Design: Impact Inferred from Temporal Change Alone

In this situation a control or reference area defined at the design or planning stage is missing. The options for suboptimal sampling design and analysis are outlined in Figure 5.6. If sampling is done at different times at a number of different locations, then temporal change in spatial pattern of the variables can be the criterion of impact. However, if there is only one location (impact site), then time-series analysis modelling must be used (e.g. Hannan, 1960, 1970; Hamon and Hannan, 1963). The most common statistical analysis model used to relate biological variables to environmental factors when there is no control area is multivariate ANOVA with canonical analysis for the multivariate case, and multiple regression and correlation for the univariate case (Figure 5.6). Green (1977, 1979) has described a detailed simulation example of a multivariate sampling design and statistical analysis for the situation where benthic samples at an impact area were spatially allocated at sites on a grid for single times before and after impact. The statistical model chosen assumed a linear additive relationship between changes in species abundance and changes in environmental variables, over a short time interval. In addition, by applying a logarithmic transformation to the data, one implies in this model that percentage change rates are linearly and additively related. The result is that spatial, within-time variation in criterion and predictor variables is removed from the analysis. Since the sampling stations are paired across time, the design is analogous to the use of a paired t-test for comparing the means of two populations where there is high within-group variation and a logarithmical pairing of individuals between the groups (Green, 1979). If sampling at all stations is repeated more than two times, the above statistical design can be easily modified. For instance, instead of using the difference in each variable between the two times, one could calculate the average change in each variable per unit time at each station. The additional sampling would allow even more sensitive tests of hypotheses regarding the effect of the impact on species abundances.

Another approach to the analysis of change in spatial pattern of species composition over a series of sampling times is to apply ordination or cluster analysis to the successive groups of data (eg. Williams et al. 1969). These methods are discussed in Sections III - 6.2.4.2 and III - 6.2.4.3 of Volume I. This analytical approach can be effectively used where an impact occurred at a certain time and location and its effects on the biological community persisted, possibly with spatial changes too.

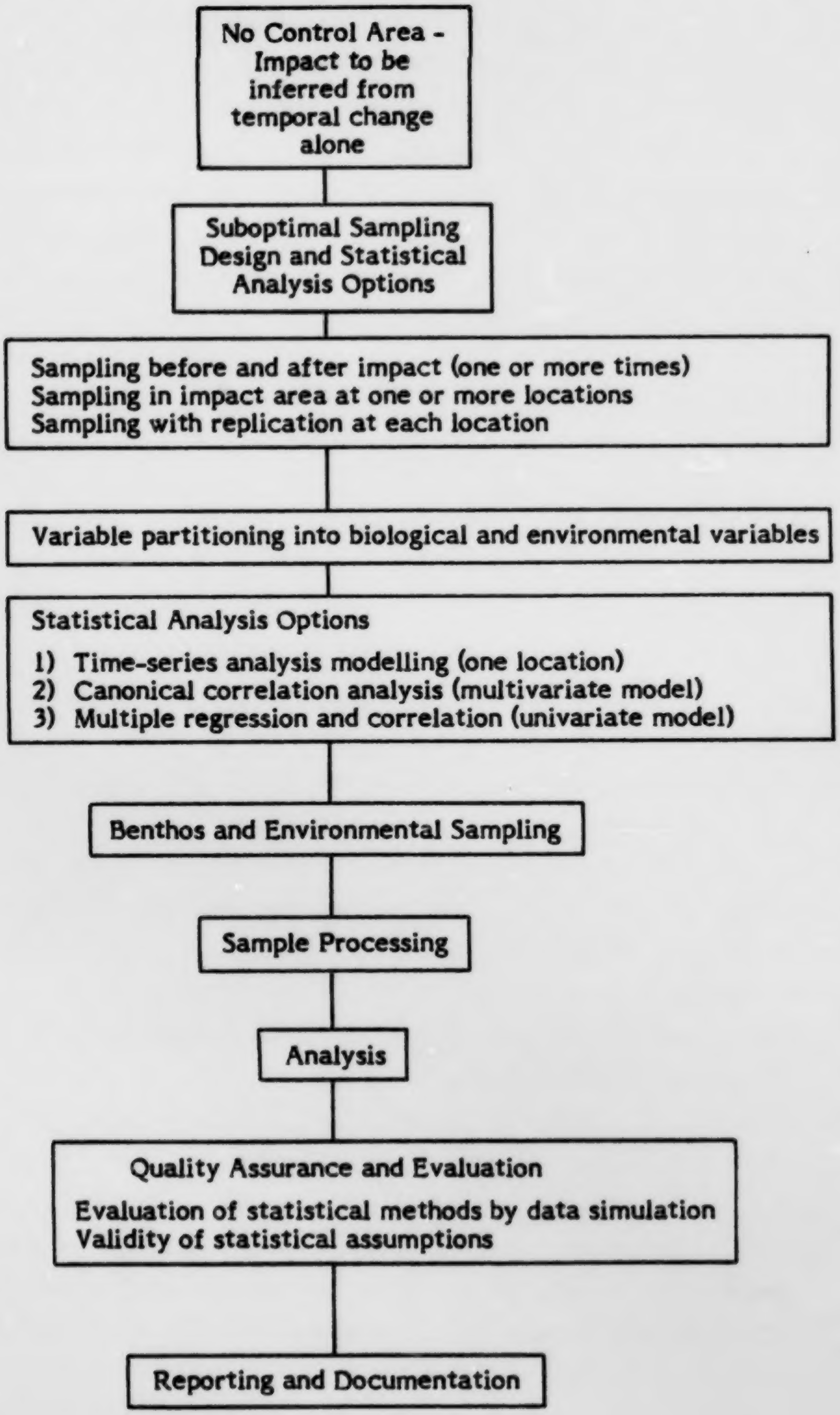

No Control Area - Impact to be inferred from temporal change alone
Suboptimal Sampling Design and Statistical Analysis Options
Sampling before and after impact (one or more times)
Sampling in impact area at one or more locations
Sampling with replication at each location
Variable partitioning into biological and environmental variables
Statistical Analysis Options
1) Time-series analysis modelling (one location)
2) Canonical correlation analysis (multivariate model)
3) Multiple regression and correlation (univariate model)
Benthos and Environmental Sampling
Sample Processing
Analysis
Quality Assurance and Evaluation
Evaluation of statistical methods by data simulation
Validity of statistical assumptions
Reporting and Documentation

Figure 5.6 Category 2: Impact Inferred from Temporal Change Alone.

Time series analysis methods (Anderson 1963, 1971; Section 5.3.1.1) can be applied in suboptimal impact studies which lack a control area if sampling is repeated several times. Time series analysis can be done with binary as well as quantitative data. Cox and Lewis (1966) and Davis (1973) have described time series analysis with binary data. A data set (for example, species counts over a series of observations), can be tested for variation in species success (presence) among times by a procedure, such as Cochran's (1950) Q-test. This test has been described by Siegel (1956) and illustrated with a worked example by Green (1979).

For a suboptimal impact study involving collection of a large set of time series species data, an option would be to test the null hypothesis that "nothing is going on in this time series of binary species data" by Cochran's Q-test, followed by ordination or cluster analysis to describe the temporal changes in the data if the null hypothesis is rejected. For instance, ordination techniques applied to data sets where the number of species greatly outnumbers the sampling times would permit the representation of temporal species-groups in a reduced space (see Section III - 6.2.4.3 of Volume I). The temporal species assemblages could be linked by arrows to show the direction of movement through the ordination space. Alternatively, cluster analysis could be applied through the method of association analysis (Williams and Lambert 1959, 1960; Green and Hobson 1970) which could be used to identify the best indicator species for further monitoring.

As mentioned in Section 5.3 (a), temporal variation (eg. seasonal or other types of cycles) should be represented in baseline and impact study models. Livingston (1976, 1977) has treated the topics of seasonal, tidal and diurnal cycles in estuarine systems as related to pollution studies. Durbin (1963) and Bulmer (1974) have described methods for estimation of seasonal and other cyclical components in time series of species data.

5.3.1.3 Baseline or Monitoring Study Designs

For studies in this group, an impact has not yet occurred and when, where or even if an impact will occur is not established (Figure 5.4). Most frequently, sampling will be done at one or more locations within one area to detect impact changes if they occur (Figure 5.7). If a control or reference area and a potentially impacted area can be established, then sampling will be performed at more than one location per area.

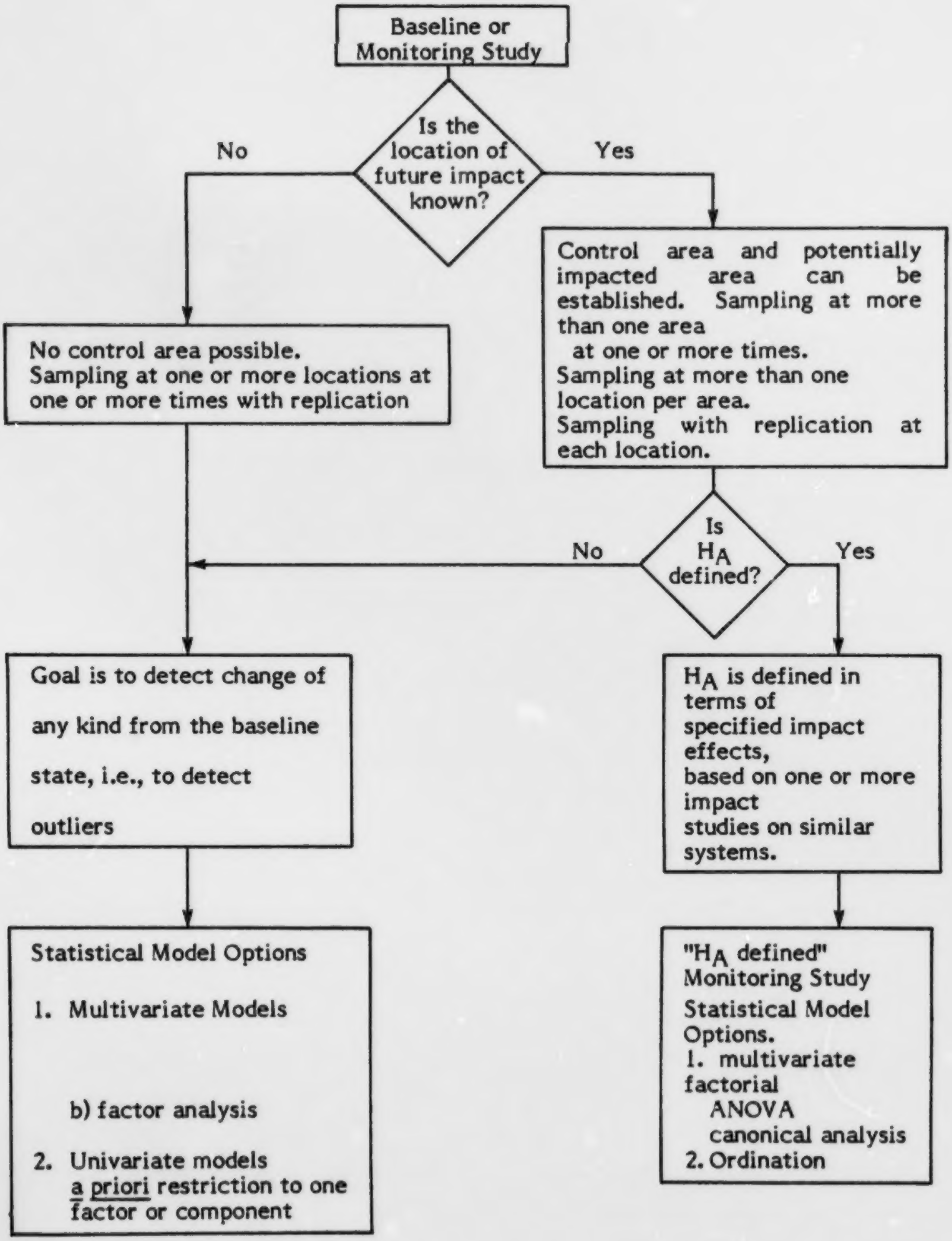

Baseline or Monitoring Study
Is the location of future impact known?
No
Yes
No control area possible. Sampling at one or more locations at one or more times with replication
Control area and potentially impacted area can be established. Sampling at more than one area at one or more times. Sampling at more than one location per area. Sampling with replication at each location.
Is H_A defined?
No
Yes
Goal is to detect change of any kind from the baseline state, i.e., to detect outliers
H_A is defined in terms of specified impact effects, based on one or more impact studies on similar systems.
Statistical Model Options
1. Multivariate Models
b) factor analysis
2. Univariate models a priori restriction to one factor or component
"H_A defined" Monitoring Study
Statistical Model Options.
1. multivariate factorial ANOVA canonical analysis
2. Ordination

Figure 5.7. Category 3: Baseline or Monitoring Study. H_A refers to the "alternate hypothesis" to the null hypothesis, H_o (Section 5.2.1).

Any monitoring study requires baseline data because impact changes can only be identified by departure from the unimpacted or reference state. The time interval and amount of detail necessary for description of the baseline state will depend on the degree of cyclical behaviour and other natural variation in that state (see Section 5.3.1.2 for a treatment of time series data).

A major decision in the design of baseline or monitoring studies is determining whether or not a specific alternate hypothesis (H_A) can be defined in terms of particular impact effects which are in contrast to the null hypothesis (H_0) that baseline conditions of the biota are unchanged (Figure 5.7).

If the biological response to the impact is uncertain, because either the nature of the impact is unknown, or it has never occurred previously, then the only appropriate H_0 is that there is no biological change to the baseline state. If this H_0 is rejected, then the aim is to detect change of any kind in the biological community from the baseline state. The various options in choosing statistical models for this situation include multivariate techniques such as ordination, factor analysis and clustering (see Section 6.2.4 of Volume I) and univariate models when there is a restriction to one pair of indicator and predictor variables in the design. The basic strategy is to detect outliers in the data which might indicate that a change from the baseline state has occurred. An example is to determine whether a new observation on species abundance belongs to the multivariate normal baseline distribution of the before impact data. An effective approach is to reduce the multidimensional data to a two-dimensional space by principal components ordination and to construct confidence ellipses (Sokal and Rohlf, 1969). The position of the new observation on species abundances relative to the confidences ellipses will indicate whether the observation is an outlier or not. Green (1979) has given a worked example of this approach.

A much more satisfactory situation for design of a monitoring study is to have an H_A defined in terms of specified impact effects. This kind of H_A requires not only information on baseline conditions, but also information about the biological response to the particular impact, based on one or more impact studies on the same or similar systems. For example, such an "H_A defined" monitoring study to detect future impacts of the same kind might be based on a previously conducted optimal impact study. Green (1979) has provided an example of a biological monitoring study based on an optimal impact study of benthic communities. He used the canonical variate associated with species interaction (CVSI) derived from canonical analysis for the biological criterion to detect future impacts of a similar type. From samples collected at several times subsequent to the impact a CVSI score was calculated from the species abundances of three species. If the estimated CVSI score exceeded a specified critical value then a possible impact would be indicated. The critical value of the CVSI score can be chosen from a cumulative percentage plot of unimpacted and impacted CVSI sample values on arithmetic probability paper (Green 1979).

5.3.1.4 Suboptimal Impact Study Design: Impact Inferred from Spatial Pattern Alone

When an impact study is initiated after the impact has occurred, then its effects must be shown and described from the spatial distributions of the biological and environmental variables (Figure 5.8). Sampling design may involve sampling at more than one location within one or more areas on one or more occasions. However, because sampling areas differing in degree of impact cannot be selected beforehand, and spatial rather than temporal pattern must be the key source of information, the most frequently used sampling design in this situation is the following:

(a) sampling on one occasion at more than one location within the impacted area, with replication at each location.

(b) samples for biological variables have corresponding observations on environmental variables.

For the statistical analysis of the spatial pattern of the variables, a two-staged procedure is recommended:

(1) reduce the biological variables (e.g. species abundances) to fewer variables in a smaller dimensional space by methods such as ordination or clustering (see Section III - 6.2.4 of Volume 1), and

(2) relate the derived biological variables to environmental variables to account for the variation in the biological variables.

Ordination procedures will reduce the biological data to a smaller number of continuous biological variables which can then be related to the environmental data

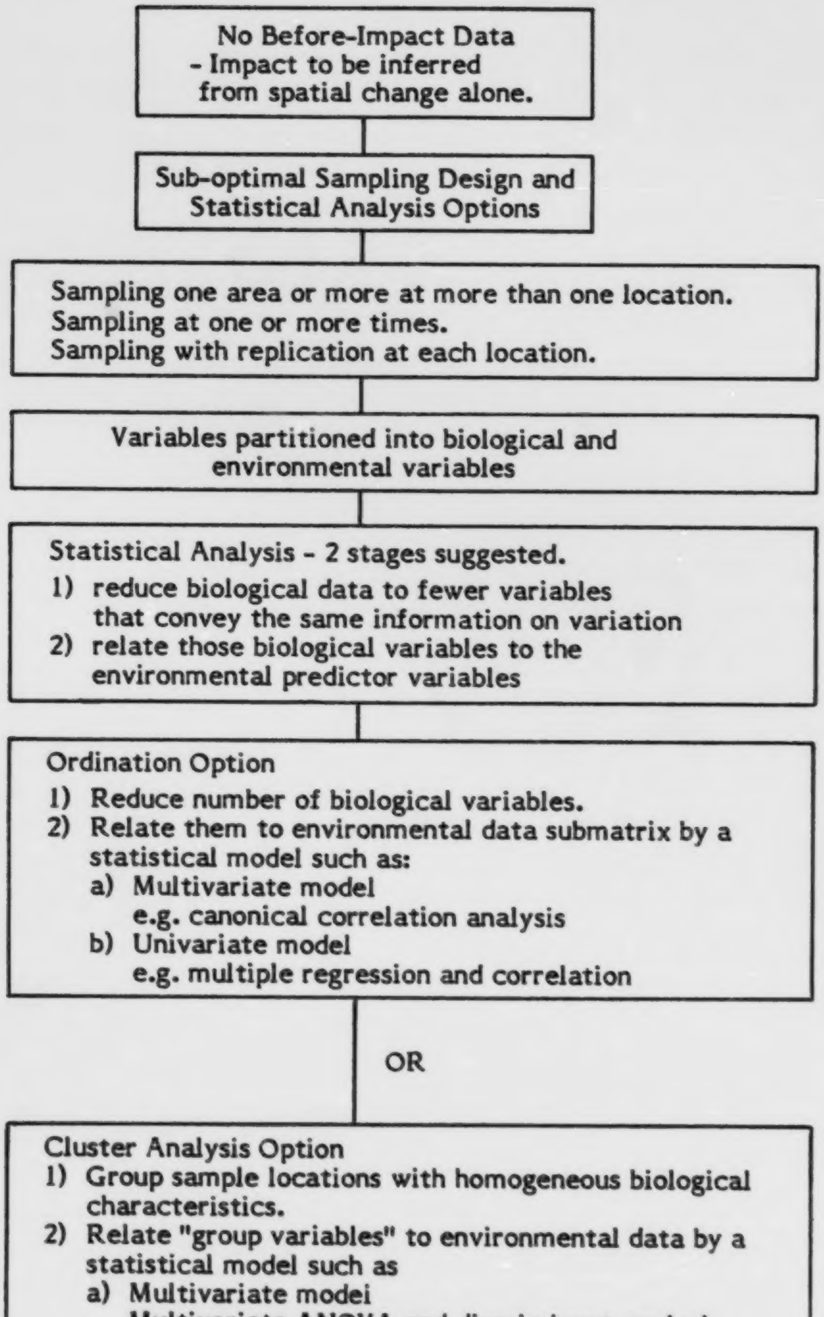

No Before-Impact Data
- Impact to be inferred
from spatial change alone.
Sub-optimal Sampling Design and
Statistical Analysis Options
Sampling one area or more at more than one location.
Sampling at one or more times.
Sampling with replication at each location.
Variables partitioned into biological and
environmental variables
Statistical Analysis - 2 stages suggested.
1) reduce biological data to fewer variables
that convey the same information on variation
2) relate those biological variables to the
environmental predictor variables
Ordination Option
1) Reduce number of biological variables.
2) Relate them to environmental data submatrix by a
statistical model such as:
a) Multivariate model
e.g. canonical correlation analysis
b) Univariate model
e.g. multiple regression and correlation
OR
Cluster Analysis Option
1) Group sample locations with homogeneous biological
characteristics.
2) Relate "group variables" to environmental data by a
statistical model such as
a) Multivariate model
Multivariate ANOVA and discriminant analysis
(or canonical analysis)
b) Univariate model
ANOVA
or c) Clustering on environmental variables too and then
relate the two cluster analyses results.

Figure 5.8 Category 4: Impact Must be Inferred from Spatial Pattern Alone.

through a statistical model. For multivariate cases, cannonical or multiple discriminant analysis (e.g., Cooley and Lohnes, 1962, 1971; Pimentel, 1978; Green, 1974, 1979) might be appropriate. In a univariate analysis, multiple regression and correlation might be applicable. Note, however, that the assumption of a linear relationship between population success and environmental factors over the natural range of the variables and during extended time intervals is often unrealistic due to the unimodal rather than linear distributions of species on environmental gradients. When using statistical models which assume linear relationships between variables (e.g., canonical correlation, factor analysis, linear or multiple regression) it is important that the linear model be applied among the environmental variables rather than among the biological variables, or between the biological variables and the environmental variables. For example, in multiple discriminant analysis (or canonical analysis), the discriminant functions used to evaluate and interpret the separation of groups of entities assume linear relationships between selected environmental variables (e.g., temperature, salinity or depth) which are logarithmically transformed if necessary. The distribution of the biotic variables (e.g., species assemblages from clustering or ordination) on the environmental variables is assumed to be nearly multivariate normal. The validity of the latter assumption, however, is not critical due to the unimodal character of biotic distributions (Green, 1979).

Another approach for relating biotic variables to environmental variables is to perform cluster analyses on the environmental factors as well as the species data (e.g., Sandilands, 1977). The results of the two cluster analyses could then be compared by methods described by Rohlf (1974), such as two-way contingency table analysis.

Whatever the methods selected for examining impact effects through analysis of spatial pattern, it must be recognized that in the absence of a before-impact temporal reference, that the inferences derived always depend on the untestable assumption that the observed spatial pattern is influenced by the impact and did not exist before the impact. In addition, results from spatial pattern analysis, especially those involving transects across environmental gradients, can be deceptive due to confounding of variables (e.g., strong intercorrelations between salinity, depth and substrate properties in transects between an estuary and open ocean; see Bird, 1970). Variation in distributions of species on environmental gradients may not be accounted for by environmental factors alone, even if all of the important ones are considered (Pielou, 1974, 1975). For example, the effect of intrinsic biological variation such as interspecific competition may produce complex species patterns along environmental gradients which could be mistakenly attributed to environmental variation in a descriptive study (Fenchel, 1975).

5.3.1.5 Category 5: When and Where Did the Impact Occur?

An applied environmental study which is initiated without knowing when or where the impact began represents the worst possible case. The aim here is to find out "when and where" and the possible degree of natural versus human causes of the changes. The approach to the problem must be to determine whether the effect existed before possible human causes were present or whether it now is evident in areas unaffected by the hypothesized human causes. The problems of mercury pollution (Katz, 1972) and chlorinated hydrocarbon residues in the food chain (Hickey and Anderson, 1968) are prime examples of impact studies of this type.

Although specific methods for study design and analysis cannot be recommended for this situation, it is noteworthy that technqiues for analysing growth rates over annual and shorter cycles have been developed for fossil shells (Panella *et al.*, 1968). The use of such methods to investigate biological effects of past environmental variation might prove to be fruitful.

5.4 Sampling Methods for Macrobenthos

Appropriate methods for collection of macrobenthos will depend on several factors, such as the target population (infauna or epifauna), substrate type (soft mud to bedrock) and sampling platform (from the ice or a research vessel). A general review of methods and recommendations for remote and direct collection of macrobenthos are presented in Sections II-3, 4 and 5 of Volume 1. Selection of a suitable sampling device is treated as a component of sampling design (Section 5.2.3). This section will consider appropriate practices for collection and subsequent treatment of quantitative macrobenthos samples.

5.4.1 Recommended Practices for Remote Collection of Macroinfauna

The following practices are advised for quantitative remote sampling of macroinfauna from soft marine sediments:

1. Select a suitable grab sampler which collects a surface area of at least 0.1 m^2 (e.g., van Veen, Smith-McIntyre: see Section II-3.31 of Volume 1 for selection criteria).

2. Determine the number of samples necessary to adequately sample the number of species present, the distribution and abundance of the infauna (see Section II-3.1.3, of Volume 1).

3. In open water, the sampling vessel should be anchored on station if possible to prevent drifting, and to reduce the wire angle so that the grab strikes the sediment surface squarely and lifts vertically.

4. Use a light wire (4-6 mm diameter) of good quality and condition so that the chance of kinking or fouling is minimized. A strong swivel (e.g., stainless steel or bronze ball-bearing type) should be connected between the grab and the wire end to prevent twisting and kinking of the wire.

5. Sampling depths should be measured by echo sounder and/or meter-block. See Rees (1971) for deck arrangements. Setting down of the grab during lowering should be done as gently as possible in order to reduce the shock wave preceding the grab. Gentle immediate closing of the grab after impact should prevent the loss of sediment due to jerky lifting of the grab before complete closure of the jaws.

6. The volume of sediment collected with each cast should be measured in a graduated container and recorded in the sampling log. If less than 5 L of sediment is obtained, as may happen on firm sand, then the results should indicate a low sample volume was collected. For a sample to be acceptable the grab should be fully closed on retrieval and should contain a full bite of sediment. Samples with evidence of leakage of contents should not be used for quantitative results.

7. Adequate training and experience in the operation of the grab should be provided for sampling personnel before actual field sampling begins. There is a "learning curve" for grab operation (see Ursin, 1954).

8. Grab sampling through the ice with a grab of sufficient size imposes many restrictions. These translate into increased sampling time (and cost) compared to open water sampling from a research vessel. Grab sampling from the ice can be done with a compact grab (e.g., short-armed van Veen,

see Petersen, 1977) by using a hand winch mounted on a portable stand. It may be possible at some locations to take samples beside leads in the sea ice (e.g. Curtis, 1972). Otherwise it will be necesssary to cut a hole in the ice which is large enough to deploy the cocked grab. Lowering the grab sideways rather than upright through the hole may be possible but is more difficult and will often result in premature tripping of the grab. Even with a "short armed" Van Veen, a hole of 80 cm will be required. A hot water drill or melter is therefore most suitable (Section 4.3.2). When replicate samples are taken, the line should be kept in different corners of the hole for each sample to prevent overlapping of grab bites. If overlapping is suspected, separate holes may be required for each grab. When sampling from a lead, previously disturbed bottom can be avoided by moving the winch and stand along the lead between samples. The choice of locations for sampling from the ice, as suggested above, will be highly dependent on ice conditions. Adverse conditions such as the presence of pressure ridges, rubble fields, open leads, or rotten ice will often dictate where sampling may or may not be done. It is, therefore, recommended that contingency plans be devised in order to satisfy the essential criteria for choice of sampling location, whether they be water depth, distance from a reference point or random positioning within a sampling grid. Benthos sampling from the ice is slow, hard work. Allowance should be made in the ice sampling schedule for several more hours per station than for open water sampling to account for transportation of gear to the station, preparing suitable size ice hole(s), and manual sampling operations under severe conditions.

5.4.2 Recommended Practices for Diver Collection of Macrobenthos

Diver-operated sampling techniques are especially suited to studies in areas with hard or gravelly substrates and uneven surfaces. On level, soft sediments diving can also be used but it has fewer advantages over remote sampling. The ability to make direct observations on the benthos and their habitat is a principal advantage, whereas relatively high cost and depth or visibility limitations are major drawbacks. Diving applications and suitable gear for cold water use are described in Section 4 of Volume I.

For quantitative diver sampling of macrobenthos the following practices are suggested:

1) For quadrat sampling, select an airlift sampler suitable for operation on the type of substrate and in the depth range to be encountered in the study area (e.g., Finnish IBP-PM (1969) suction sampler for level hard substrates, in 2-15 m of water; see also Section 5.2.3 of this guide and Section 4.1.41 of Volume I for details and references on the equipment available and their modes of operation). For shallow (< 20 m) study areas in which both hard substrates and firm sandy sediments occur, it would be desirable to equip an airlift tube with the funnel of the Finnish sampler for the hard substrates and the coring tube of the Barnett and Hardy (1967) sampler for sandy or soft sediments.

2) Collect the benthos samples as indicated in the cited descriptions, keeping replicate samples separate for processing and analysis. To achieve this, easily detachable sample collectors or more than one sampler may be used to minimize loss of working time between collection of replicate samples.

3) If line transects are used to sample a study area, then the techniques of *in situ* counting, quadrat sampling and quantitative diver operated still photography may be used in conjunction with the transect to representatively sample the range of macrobenthos present (see Section 4.1.4.1 of Volume I).

5.4.3 Dredging and Trawling

Dredges and trawls are generally used for collecting epibenthos and demersal fish, cephalopods and crustaceans associated with or feeding on the sea bottom. General references on these samplers are Gunter (1957), Holme (1964) and Holme and McIntyre (1971). Section 3.2.1 of Volume I presents a review of these devices and their modes of operation. **Dredging and trawling in arctic waters are restricted to open water in areas relatively free of floating ice.**

The following practices are advised for sampling by biological dredge:

1) A weak link which breaks first under heavy strain due to a hangup on the bottom should be used to prevent loss of gear and to improve safety of operation (see Section 3.2.1 of Volume I).

2) The dredge should be towed slowly and steadily (0.5 to 1 m/sec) on a single 11 mm diameter wire rope of 6 x 19 construction in good quality and condition. A strong swivel should be placed between the dredge and the wire terminal to prevent twisting and kinking of the wire.

3) The surface area covered by the dredge is usually estimated from the length of time at the bottom depth multiplied by the speed of the tow and by the width of the dredge. Some recent dredges have metering devices to record the distance moved along the bottom (e.g., Martec/Shell).

For sampling macroepifauna by trawl, the following procedures are suggested.

1) The bottom topography of the study area should be examined for possible obstructions or hazards by consulting bathymetric charts or by running echo sounding transects along the routes of proposed sampling tows.

2) **A beam trawl with a 3-m mouth aperture is recommended over the otter trawl** for this purpose since the net gape is fixed by the beam whereas the spread of the otter boards and net wings is quite variable for the otter trawl. The mesh size of the net should be appropriate for retention of the target organisms.

3) The beam trawl should be towed at 1 m/sec from a single wire rope (11 mm diameter, 6 x 19 construction) of good quality and condition. A sturdy swivel should be inserted between the trawl bridle and the terminal of the timing warp to prevent twisting and kinking of the wire.

4) A bottom time of 10 minutes per tow and two tows per location are suggested for obtaining representative samples of the epifaunal species in most areas. Trawls equipped with odometers to record the distance travelled while on the bottom are advised for quantitative collections of epifauna (e.g., Carney and Carey, 1980).

5) Care should be taken to remove epifauna from net meshes as well as from the cod end of the net.

5.5 Treatment and Processing of Macrobenthos Samples

A scheme for the selection of appropriate methods for treatment and processing of macrobenthos samples is presented in Figure 5.9. General references for the treatment of zoobenthos samples are Thorson (1957), Birkett and McIntyre (1971), Stirn (1981) and Eleftheriou and Holme (1984). Techniques of collection and preservation of invertebrates for taxonomic purposes are described by Lincoln and Sheals (1979). A review of methods is given for zoobenthos, Section III-6, Volume 1, and for phytobenthos in Section II-5, Volume 1.

5.5.1 Recommended Practices for Treatment of Samples of Macroinfauna

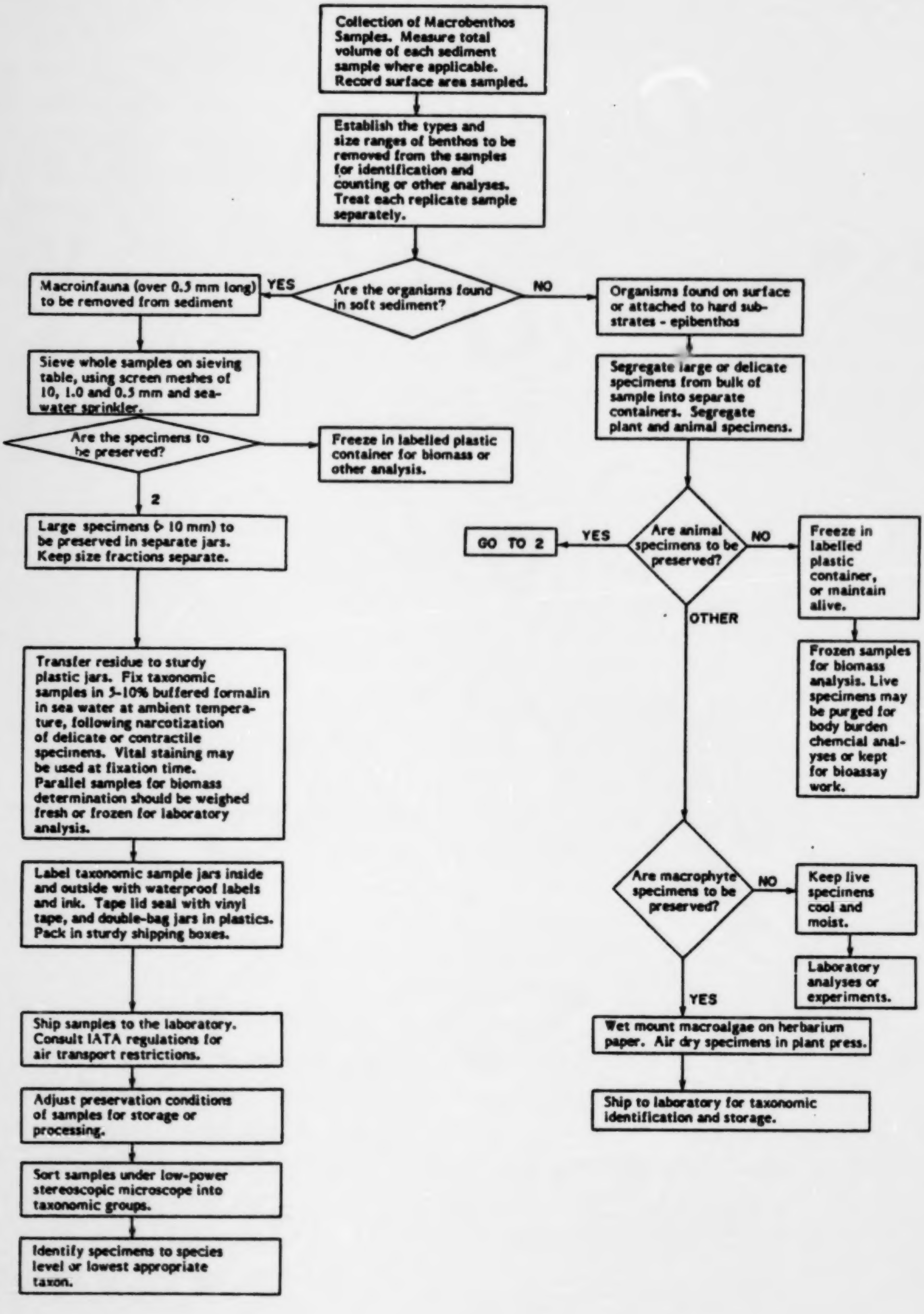
The following procedures for handling macrobenthos samples collected by grab are recommended:

1) The total volume of each sediment sample should be measured in a calibrated container. The surface area sampled should also be recorded. If subsamples are removed (e.g., by a small corer), then the surface area and volume removed should be indicated.

2) Each replicate sample should be separately sieved and fixed in the field on the day of collection (i.e., as soon as possible after sample collection). They should also be separately sorted and enumerated for species composition and abundance (see Section 5.2.3). **It is impossible to sort benthos out on the ice in cold weather unless adequate heated space is available. It is usually necessary to transport samples back to a camp. It should be remembered, however, that 50 litres of mud may weigh up to 100 Kg. The extra weight of several samples may impose transport restrictions.**

3) The extraction of macrofauna should be done (at a minimum) by sieving the whole sample on a sieving table or hopper with a 0.5 mm mesh sieve and the aid of a sea water sprinkler. A series of mesh sizes, 0.5, 1.0 and 10 mm is suggested to improve size fractionation by separating coarse mineral or detrital particles and large benthos specimens from the finer fractions. The extraction and sorting of benthos from size fractionated samples is easier and

Figure 5.9 Selection of Suitable methods for macrobenthos sample treatment and processing


Collection of Macrobenthos Samples. Measure total volume of each sediment sample where applicable. Record surface area sampled.
Establish the types and size ranges of benthos to be removed from the samples for identification and counting or other analyses. Treat each replicate sample separately.
Are the organisms found in soft sediment?
YES
NO
Macroinfauna (over 0.5 mm long) to be removed from sediment
Organisms found on surface or attached to hard substrates - epibenthos
Sieve whole samples on sieving table, using screen meshes of 10, 1.0 and 0.5 mm and sea-water sprinkler.
Segregate large or delicate specimens from bulk of sample into separate containers. Segregate plant and animal specimens.
Are the specimens to be preserved?
Freeze in labelled plastic container for biomass or other analysis.
2
Large specimens (> 10 mm) to be preserved in separate jars. Keep size fractions separate.
GO TO 2
YES
Are animal specimens to be preserved?
NO
Freeze in labelled plastic container, or maintain alive.
OTHER
Frozen samples for biomass analysis. Live specimens may be purged for body burden chemcial analyses or kept for bioassay work.
Transfer residue to sturdy plastic jars. Fix taxonomic samples in 5-10% buffered formalin in sea water at ambient temperature, following narcotization of delicate or contractile specimens. Vital staining may be used at fixation time. Parallel samples for biomass determination should be weighed fresh or frozen for laboratory analysis.
Label taxonomic sample jars inside and outside with waterproof labels and ink. Tape lid seal with vinyl tape, and double-bag jars in plastics. Pack in sturdy shipping boxes.
Are macrophyte specimens to be preserved?
NO
Keep live specimens cool and moist.
Laboratory analyses or experiments.
YES
Wet mount macroalgae on herbarium paper. Air dry specimens in plant press.
Ship samples to the laboratory. Consult IATA regulations for air transport restrictions.
Ship to laboratory for taxonomic identification and storage.
Adjust preservation conditions of samples for storage or processing.
Sort samples under low-power stereoscopic microscope into taxonomic groups.
Identify specimens to species level or lowest appropriate taxon.

more convenient than from unfractionated samples. Separation of bulky objects or specimens with complex surfaces at the time of collection or sieving will reduce some of the difficulties in subsequent sorting of the smaller organisms. The large objects removed should be inspected for small organisms on their surfaces.

4) All organisms and other residue on the screens should be transferred to suitable containers. Jars and lids made of sturdy plastic are more resilient than glass jars for shipping. Specimens for other analyses such as chemical body burden measurements should be collected in separate grab hauls and prepared according to procedures recommended for specific types of analyses (metals or organics; see for example Section 6.2.3 and 7.3.2).

5) The organisms should be fixed in a buffered solution of 5-10% formalin in sea water at ambient temperature (following narcotisation where appropriate, Section II-6.1, Volume 1). Borax, hexamine, or marble chips may be used to buffer the formalin against acidification. For thorough fixation, the volume of formalin to sample residue should be in a ratio of about 4:1, and the contents should be well mixed. Vital staining (e.g., with Rose Bengal) may be used at the time of initial fixation to aid in sorting. See Williams and Williams (1974) for details on vital staining.

6) Permanent waterproof labels made of paper with high rag content should accompany each sample or subsample in the container throughout all stages of processing, shipping and storage. The labels should include pertinent sampling information and sample identification. The labelling materials (paper, ink) should be inert so that interference with other analyses is avoided (e.g., ball-point ink interferes with hydrocarbon analyses). Labels on the jar lids and sides should be in addition to, rather than in place of, labels inside the containers. A suggested list of information for inclusion on labels is given in Section III-6.1.1, Volume 1.

7) Detailed accounts of sampling information should be recorded in a field sampling log book. Prepared log sheets with spaces for recording pertinent data and other types of observations or samples taken at the station are convenient for this purpose. This information should be retained in permanent files for later reference and analysis of data.

8) Before shipping to the laboratory, jars containing formalin should be sealed with vinyl tape and double-bagged in polyethylene plastic to prevent leakage of contents, and secured with packing in sturdy shipping boxes. **Formaldehyde is a restricted item for air transport.** Concentrated formaldehyde should be shipped by surface transport in sturdy leakproof containers. Samples containing dilute formalin may be shipped by commercial air carriers if properly packaged, labelled and declared as hazardous goods according to IATA regulations.

9) After shipment to the laboratory and a fixation period of 1 week to 1 month, the formalin-preserved specimens for taxonomic identification should be transferred to 70% ethanol or isopropanol to prevent decalcification of delicate calcareous structures and to reduce the exposure of taxonomists to formaldehyde fumes.

5.5.2 Recommended Treatment of Samples of Epibenthos

Samples of epibenthos from dredges, trawls and airlift samplers can be handled according to the procedures below if sieving is not required to separate the organisms from sediment. Samples requiring sieving can be treated in the same way as samples of macroinfauna (section 5.5.1).

1) For determination of the total wet-weight biomass of macroepifauna, the sample in the cod end of the trawl, dredge or airlift can be transferred into a tared mesh bag, drained, freed of detritus, blotted to remove surface moisture and weighed on a top-load balance (see Section III-6.2.1, Volume I).

2) The sorting, as close to species level as feasible or at least to separate plant and animal specimens, should be done in the field. The faunal component of the sample can be preserved as in Section 5.4.1. Specimens to be used for chemical body burden analyses should be treated according to the recommendations for the specific type of analysis (see for example Section 6.2.3 and 7.3.2). Representatives of each species used for various analyses should be preserved for species identification. The numbers of specimens of each species removed from a quantitative sample should be recorded to permit their inclusion in species enumeration.

3) Preservation of macroalgae for species determination is generally done by wet mounting the algal specimens on herbarium paper and air drying them between layers of wax paper and newsprint in a plant press. The layers of waxpaper and newsprint should be changed frequently in the first few hours of drying. Macrophytes to be kept alive for laboratory analyses or experiments should be kept cool and moist. A general reference on phycological methods is Stein (1973).

5.5.3 Sorting and Analysis of Macrobenthos Samples

Benthos sample sorting is reviewed in Section III-6.1.2, Volume I. Procedures which help to minimize sorting errors are given below:

1) The use of low-power stereoscopic microscopes (e.g., Wild M-5 or equivalent) and channelled counting trays with background grids (e.g., Schlieper, 1972) greatly increases the thoroughness of finding small specimens in samples.

2) The use of bulk vital staining (e.g., Rose Bengal) makes small organisms easier to distinguish from debris (Section 5.5.1, No. 5).

3) The efficiency of sorting can be checked by quality control procedures (Section III - 6.2.3, Volume I) and by examining the distribution of individuals among the species present for deviations from a log-normal distribution. As indicated by Gray and Mizra (1979) such deviations may result from the failure to find rare species or from the inaccurate identification of species.

5.6 Analysis of Macrobenthos Samples

5.6.1 Estimation of Biomass

Biomass, or standing crop, can be defined as the amount of living substance making up the population of organisms under study. Usually it refers to the weight of a sample population which was obtained from a sampled surface area or volume. For comparibility and convenience, biomass of benthos is generally reported on a per m^2 basis, and is calculated from the mean of replicate samples from a station or group of stations. The choice of units of biomass usually reflects the method for its estimation, which can be in terms of wet weight, whole or decalcified dry weight, or ash-free dry weight, carbon and/or nitrogen content or caloric (energy) equivalent.

5.6.1.1 Wet-Weight Biomass

Wet weight is the easiest, but least reliable measurement of biomass. The following practices are advised for this approach to biomass estimation:

1) Use of unpreserved specimens is best, since preservation generally results in significant loss of organic material (see Section III-6.2.1.1, Volume I) and changes in length-weight relationships.

2) Wet weighing can be done in the field on a top-load balance unless samples are so small that an analytical balance is needed for adequate precision. Specimens should be free of sediment and detritus, and blotted to remove surface moisture. See Howmiller (1972) for an alternate laboratory method for removing surface water from small organisms by centrifugation.

3) The removal of shells and other hard parts from large specimens is not advised unless it can also be applied to smaller species or individuals. If this approach is used, though, it should be clearly stated.

4) If specimens cannot be weighed in the field, an alternative method is to collect a parallel series of samples which can be frozen and used for laboratory determination of wet (and dry) biomass.

5.6.1.2 Dry Weight

The dry biomass is the weight of completely dehydrated specimens of the sampled population. There are three main ways of removing water from the tissues:

(a) heating in an oven at 70-100°C, atmospheric pressure;
(b) desiccation under vacuum at temperatures up to 60°C;
(c) freeze drying or lyophilisation

Provided that the dry tissue is not needed for other analyses and that enough time is allowed for complete drying, all three of these methods will give similar results. The following practices and precautions are recommended, where applicable:

1) A test run should be made to check the minimum time for complete dehydration (constant weight) before a large number of measurements is started with a given drying device. This may be necessary because larger organisms require longer to dry to constant weight than small ones.

2) If an oven is used, temperatures should not go over 100°C, or losses of volatile tissue substances may result. Samples should be cooled to room temperature in a desiccator before weighing.

3) Vacuum drying at 60°C takes a relatively short time, denatures enzymes which may cause autolysis at room temperature, and does not result in serious volatilisation losses. Specimens dried this way are also suitable for colorimetry or dry-ashing. See Crisp (1971, 1984) for further details on these procedures.

4) If dry-ashing is to be done as well, the same dishes (silicon or heat resistant crucibles) should be used for both procedures to save time.

5) Lyophilisation is the gentlest drying method, in general, and is advised if further chemical analyses are to be performed on the samples.

6) Dried samples are best stored either sealed from moisture in a deep freeze or under continuuous desiccation at room temperature.

7) Top-loading balances may be adequate for weighing large dry macrobenthos samples. For small samples, analytical balances (with ±1% reproducibility) may be used. A microbalance is needed for very small samples.

8) For all dry biomass procedures, specimens must be free of sediment or detritus and sea water. A rinse in distilled water (for up to 30 minutes) is advised prior to desiccation.

9) Calcareous exoskeletons may be removed by hand or by decalcification

treatment if the practice is applied consistently to all taxonomic groups. See Humason (1965) pp. 29-31 or Section III-6.2.1.2, Volume 1 for details on decalcification. **The ashing procedure (Section 5.6.1.3) produces more accurate biomass results than decalcification.**

10) It is critical that every drying procedure be carried out under identical conditions if seasonal surveys are conducted in a given area. This precaution prevents systematic variation in drying efficiency which may be mistakenly interpreted as a seasonal effect.

5.6.1.3 Ash-free Dry Weight

The ash content of the benthos samples, which is made up of inorganic substances such as salts, silica, calcium carbonate and phosphate, is determined after dry weighing by incinerating the organic substances in a muffle furnace. References on measuring ash weight are Crisp (1971, 1984) and Stirn (1981). **The following practices and precautions are recommended:**

1) Samples can be dry weighed in tared crucibles suitable for the ashing procedure.

2) To ensure complete removal of the organic matter, an incineration period of four to six hours at 500-600°C is advised.

3) It is desirable to use equipment with stable thermoregulation and a thermorecorder.

4) When incineration is completed, the samples can be cooled to about 80°C in the open, and then to room temperature in a desiccator, before they are weighed on an analytical balance or microbalance.

5) Ash-free dry weight is calculated by subtracting the ash weight from dry weight. It is a reliable estimate of total organic matter for samples containing mainly soft-bodied organisms.

6) For samples with a high proportion of organisms with calcareous exoskeletons, the ash content is seriously underestimated by the above procedure

due to significant volatilisation of inorganic carbon dioxide from carbonates and hydrated water from silicates. Therefore, groups such as molluscs, barnacles and echinoderms should be treated differently in order that the dry organic weight of the soft tissues and exoskeleton can be measured separately. Crisp (1971) has described techniques appropriate for the various groups involved.

5.6.1.4 Estimation of Biomass in Terms of Nitrogen Content

An alternative to estimating biomass as ash-free dry weight is to chemically analyse the organic tissue for nitrogen content, which is assumed to be proportional to the amount of living matter. A review of methods based on nitrogen analysis is presented by Giese (1967). Details of the classical Kjeldahl measurement of nitrogen are given by Barnes (1959. pp. 148-150). It is recommended that results obtained by this procedure be reported as biomass (mg Kjeldahl nitrogen) rather than as biomass (mg total nitrogen).

5.6.1.5 Measurement of Caloric Content

The caloric content of any substance is defined as the energy released during complete combustion of one gram of the dry substance. The caloric content of a dry tissue homogenate from a whole organism is an important factor in biological production studies for converting dry biomass into energy units. Seasonal changes in caloric content must be allowed for when converting dry weight into energy, due to changes in energy content between life-history stages of many organisms.

References on detailed procedures for measuring caloric content are Phillipson (1964) for calorimetry and Hughes (1970) for the iodate oxidation method. Crisp (1971, 1984) has reviewed calorimetry and chemical methods of measuring caloric content of organic matter.

5.6.2 Taxonomic Identification and Enumeration

The analysis of benthic communities is based on accurate identification and enumeration of specimens in benthos samples. A comprehensive study of benthos requires a team of good taxonomists for the identification of the major taxonomic classes, and access to outside expertise for assistance in identification of the remaining biota. A marine benthic research team should include specialists for the important taxonomic groups such as polychaetes, crustaceans (e.g. amphipods, isopods, and cumaceans), molluscs and echinoderms. Some groups which are usually represented by a small number of easily recognizable species, can be identified by non-specialists. Outside specialists may be needed for taxa which may be unfamiliar to the members of the team, such as bryozoans, sponges and ascidians. Mayr (1969) and Gosner (1971) have described the types of taxonomic keys and have provided introductions to the practices of systematics and taxonomic identification.

Specimens of the major taxonomic groups may be identified to species or temporarily coded in situations where a species is recognized but the positive identification needs further work. The abundance of all species present in the sample can be counted and recorded either during the identification process or afterwards. Only specimens which were viable at sampling should be included, not skeletal or other remains. In the case of damaged specimens, only the head segments are counted as specimens, not appendages or other body parts. References used in the identification of specimens should be cited, at least in the initial reporting of results.

5.7 Quality Control in Benthos Studies

The purpose of a quality assurance program for a measurement system is to reduce errors to tolerable limits and to ensure that the measurements are likely to be of acceptable quality (Taylor, 1981). Quality control is the mechanism within a quality assurance program which controls errors, while quality assessment is the means to verify that the system is operating within acceptable limits. Since quality is subjective and varies according to situation, tolerable limits of error must be determined on the basis of cost-benefit considerations for each property to be measured. Quality control in benthos studies is complicated by the fact that true values of parameters such as species density and biomass for natural populations are unknown. Therefore, it is impossible to determine the "accuracy" of the methods used (i.e. how close the estimates are to the true values). Precision (the relative difference between replicate or repetitive measurements) of benthos estimates can be established, however.

Measures for quality control which are applicable to benthos studies (e.g. Stirn, 1981; Tetra Tech, 1982) include:

1) Good laboratory practice, which requires that:

   a) proper procedural protocols are written and observed in practice. These include sampling protocol, steps for quality control in the field, methods of sample treatment, methods of analysis, quality control steps in the laboratory and standardized calculation and data reporting;
   b) high standards of personnel training and laboratory management are used; and

   c) field and laboratory procedures are inspected and audited by a third party.

2) Field sampling quality control including:

   a) collection of an adequate number of samples;
   b) replication to the extent possible of a fixed percentage of samples;
   c) preparation of detailed written sampling directions;
   d) documentation of sampling in field log books;
   e) verification of the number, type and location of samples obtained;
   f) careful selection, use and maintenance of sampling equipment;
   g) adequate inspections to guard against inadequate sample preservation, leakage or loss during collection, transfer, shipment and storage;
   h) adequate chain of custody and labelling for samples; and
   i) collection of reference (background) and contingency samples.

3) Laboratory quality control:

   a) preparation of detailed written protocols for sorting and identification;

   b) a fixed percentage (e.g. 5%) of all samples should be resorted by individuals different from those who conducted the original sorting. This should be a double-blind test. Results of the second sorting should be maintained.

   c) a fixed percentage (e.g. 5%) of the species identified by each member of the taxonomic team should be verified by a competent independent taxonomist. Results of the verifications should be maintained.

d) complete records or log sheets of sorting, processing and laboratory analysis of each replicate sample should be maintained in a file with sampling data.

e) a voucher or laboratory museum collection of specimens which are representative of each species (or lowest taxon) collected during benthos surveys in a study area should be prepared and maintained. Efforts should be made to have the identifications verified and representative specimens deposited with a public museum collection whenever possible (e.g. National Museum of Natural Sciences). Contacts in the National Museum of Natural Sciences, Ottawa, Ontario, K1A 0M8 are listed in the Directory of Marine and Freshwater Scientists in Canada; Anon (1984). The laboratory museum collection should be maintained in 70% ethanol or isopropanol according to appropriate procedures (Levi, 1966). This collection is invaluable for reference and verification purposes.

f) comprehensive and updated taxonomic literature should be maintained in the laboratory for reference during the identification process.

g) inter-laboratory comparison studies may be conducted to establish or verify the level of performance of a laboratory or taxonomist and to provide a comparison of proficiencies of participating laboratories. For example, Ellis and Cross (1981) have described a protocol for inter-laboratory comparisons of biological species identifications or "Ring tests".

Since precise taxonomic identifications are necessary for reliable analyses of communities, it is recommended that a skilled team of taxonomists and collaborating specialists be employed, and appropriate guidelines for quality control observed.

6. METHODS FOR THE ANALYSIS OF METALS IN SEDIMENT, TISSUES AND INTERSTITIAL WATER

6.1 Introduction

The purpose of this section is to provide assistance in choosing methods to study metal concentrations. It is based on the information generated by the Review of Methods (Volume I). The present chapter attempts to develop guidelines for selecting those methods which are appropriate for specific purposes.

For example, an investigator may wish to accurately determine background metal concentrations, anomalous concentrations or those above legally specified limits, e.g. the O.D.C.A. limits for Hg and Cd. An environmental assessment may require the determination of only two particular elements or a broad scan of what can be detected. The objective may be to measure the total metal concentration, some fraction thereof (e.g., the "biologically available" fraction, or some particular metal species (e.g. methylmercury). The number of samples to be analysed may be large or small, and the accuracy and precision required is variable.

Three types of environmental samples are considered:

1. **sediment interstitial waters,**
2. **whole sediments, and**
3. **benthic organisms.**

The analysis of these samples for metals follows the general outline - sampling and pretreatment, digestion, preconcentration and instrumental quantification. Sediments and tissues must be digested to produce a solution that is suitable for instrumental quantification by these techniques; digestion is not essential for interstitial waters. The various factors to consider are outlined in Figure 6.1.

Three instrumental techniques are considered:

1. **flame atomic absorption spectrometry (F-AAS),**
2. **graphite furnace atomic absorption spectrometry** (GF-AAS) and
3. **inductively coupled plasma atomic emission spectrometry (ICP-AES).**

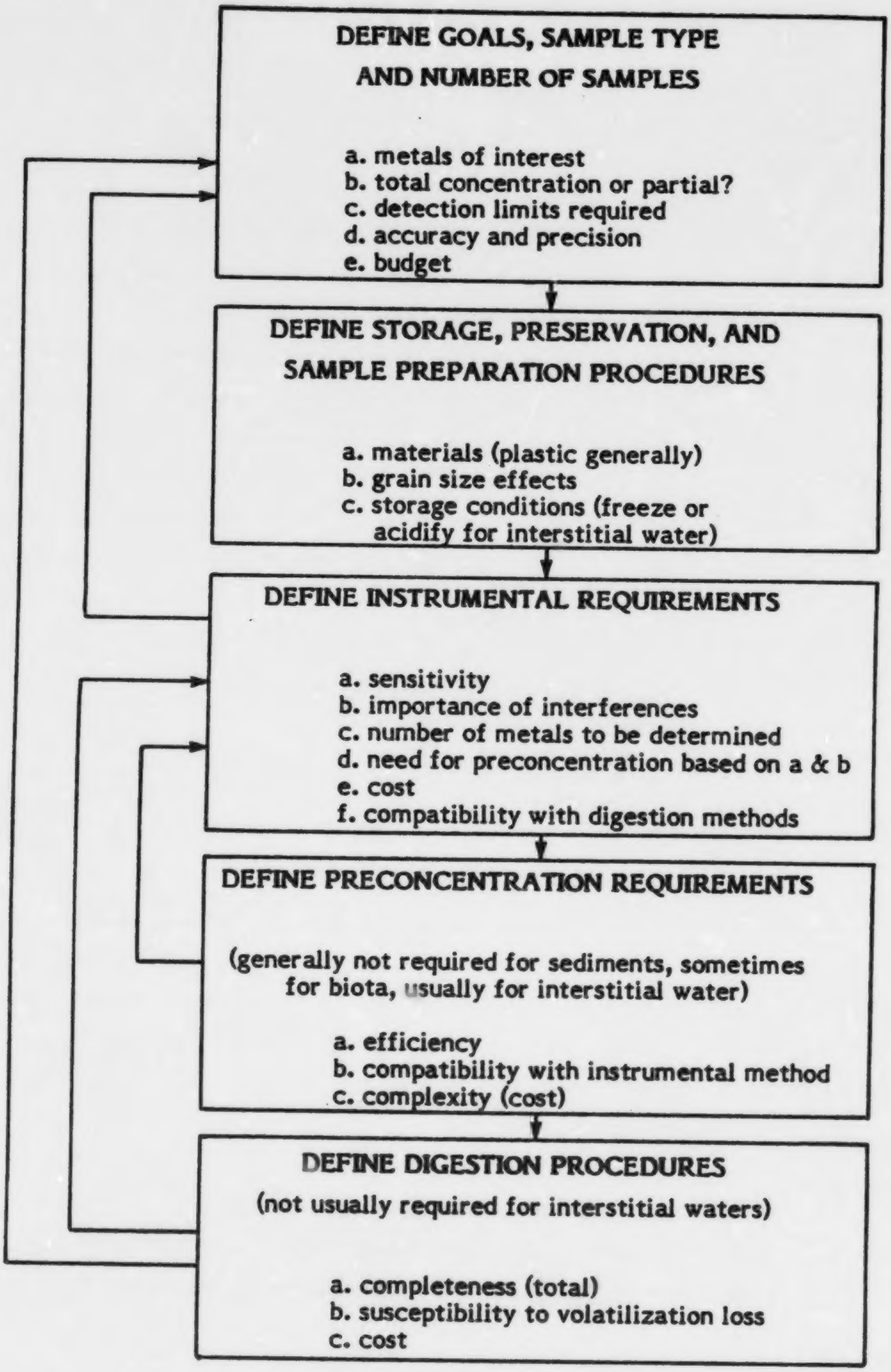

DEFINE GOALS, SAMPLE TYPE AND NUMBER OF SAMPLES
a. metals of interest
b. total concentration or partial?
c. detection limits required
d. accuracy and precision
e. budget
DEFINE STORAGE, PRESERVATION, AND SAMPLE PREPARATION PROCEDURES
a. materials (plastic generally)
b. grain size effects
c. storage conditions (freeze or acidify for interstitial water)
DEFINE INSTRUMENTAL REQUIREMENTS
a. sensitivity
b. importance of interferences
c. number of metals to be determined
d. need for preconcentration based on a & b
e. cost
f. compatibility with digestion methods
DEFINE PRECONCENTRATION REQUIREMENTS
(generally not required for sediments, sometimes for biota, usually for interstitial water)
a. efficiency
b. compatibility with instrumental method
c. complexity (cost)
DEFINE DIGESTION PROCEDURES
(not usually required for interstitial waters)
a. completeness (total)
b. susceptibility to volatilization loss
c. cost

Figure 6.1 Outline of Factors to Consider in Choosing a Method of Analysis for Metals in Sediments, Biota or Interstitial Waters.

Because the instrumental techniques have different sensitivities and the sample types have different concentration levels, the type of sample to be analyzed affects the choice of instrumental technique. Flame AAS and ICP do not have sufficient sensitivity to analyse interstitial waters without preconcentration, while GF-AAS can sometimes analyse interstitial waters without preconcentration. Sediments and tissues are usually analysed by F-AAS and ICP, reserving GF-AAS for the more difficult metals. Sample type also affects the need for preconcentration. Preconcentration is almost ubiquitous for interstitial waters, common for tissues and rare for sediments.

Sample type determines the method of digestion that is employed. Complete dissolution of sediments requires HF plus oxidizing acids. Alternatively, alkaline fusion with acid dissolution of the flux can be used. Tissues require a more oxidizing mixture of acids and HF is usually not needed. Alternatively, tissues can be first ashed in a high or a low temperature (atomic oxygen) furnace. Interstitial waters are not digested unless it is thought that metal-organic complexes or organometallic compounds might interfere with the preconcentration. Digestion of interstitial waters can usually be accomplished under milder conditions than those needed for tissues.

The metals differ in their chemical properties and this affects the choice of digestion and preconcentration methods. The metals also differ in their detection limits on a given instrument. Rather than develop individual methods for each metal, chemists usually try to devise general methods for groups of metals to take advantage of similarities in behaviour. Multi-element methods often result in a considerable time savings. **For convenience, the following metals are considered as a single group: Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn. These elements will be referred to as Group I metals in the text that follows.** Many of the methods for metals of this group are multielement methods which include three or more elements.

Arsenic and mercury are special cases because of their volatility, their formation of organometallic compounds and their extremely low concentrations. Also, special preconcentration techniques are available for them which are superior to other techniques in sensitivity and freedom from interferences. Mercury is unique in that it is nearly always determined alone by cold vapour atomic absorption spectrometry (CV-AAS). The technique involves reduction of mercuric ions to elemental mercury which is separated from the solution as a vapour. For arsenic, the hydride generation (HG) technique is similarly important. Arsenic can also be determined in combination with other hydride forming elements by HG-AAS or HG-ICP.

To facilitate comparison and evaluation the methods have been broken down into their component parts. In preparing a plan it is usually necessary to consider these factors in this order and this is how they have been presented in this Guide.

1. **sampling and pretreatment.**
2. **instrumental quantification,**
3. **preconcentration,**
4. **digestion,**

The two general criteria that have been used to evaluate the methods are performance and cost. Assessing the level of performance and cost of the methods being compared is usually not easy. Performance can be measured by the limit of detection, accuracy and precision. The quality and quantity of information given by methods reports on these quantities varies. There are very few experimental studies where different methods have been objectively compared. Specific information on costs is generally lacking in methods reports, but the factors affecting cost (i.e., capital costs, sample throughput and level of skill required) can usually be assessed. Therefore, due to the limited nature of the information available from the methods reports, the comparison and evaluation of the methods will necessarily be somewhat subjective.

6.2 Sample Preparation and Sample Preservation

6.2.1 Sampling and Sample Preparation Methods for Interstitial Waters

Due to the extremely low concentrations of trace metals, the sampling and storage of interstitial water can be very problematical from the standpoint of contamination. Special techniques that are required for seawater analysis, such as the use of clean room facilities, special purification procedures for container surfaces and ultra-pure reagents should be applied equally to interstitial waters. These techniques have been discussed by Batley and Gardner (1977) and Erickson (1977). Also, techniques that are applicable to specific metals are usually given in papers describing the analytical methods for those metals (e.g. Bloom and Crecelius (1983) for mercury, Andreae (1977) for arsenic). In addition, careful attention must be given to the procedures used to collect sediment samples and to extract interstitial water from them, not only to avoid contamination but also to avoid creating other artifacts. For example, changes in concentration or speciation might result from exposure to air or temperature change before or during extraction of interstitial water from sediments. The safest procedures are those which extract and preserve samples in conditions as close as possible to the natural ones. The paper by Kruikov and Manheim (1982) discusses the problems involved in sampling interstitial waters.

There are two commonly used techniques for extracting interstitial water from sediments - centrifugation and low pressure squeezing. Both techniques require that a fresh, representative, uncontaminated sample of whole sediments be obtained, usually by grab or core sampler (Section 3.2). The in situ extraction techniques, though they should in principle subject the sediment-water system to less perturbation, are usually impractical in the Arctic and are therefore excluded from this treatment. Batley and Giles (1980) tested three centrifuge techniques and low pressure squeezing for efficiency. For sandy sediments, solvent displacement centrifugation gave the highest recovery; while for clay sediments, low pressure squeezing and solvent displacement centrifugation were about equally effective. The solvent displacement centrifugation technique described by Batley and Giles (1980) utilizes a heavy, water immiscible solvent, 'Fluoriner FC-78', to displace interstitial water from the sediments during centrifugation. The low pressure squeezing apparatus described by Presley et al.(1967) is suitable for trace metal work.

A low pressure squeezer may be able to produce a larger quantity of interstitial water in less time than centrifugation, but centrifuges are more common than low pressure squeezers. Both techniques can be applied on board a research vessel. On the average, it appears that the low pressure squeezing device of Presley et al. (1967) and the centrifuge methods of Batley and Giles (1980) are about equally suitable for extracting interstitial water samples from arctic sediments for trace metal determinations.

6.2.2 Sample Preparation Methods for Sediments

The procedures used for sample storage and preparation for analysis depend on the kind of determination that is to be performed. Figure 6.2 outlines typical sequences for the main kinds of determination. After the grab or core sampler has been retrieved, a representative subsample is obtained with a suitable implement (e.g. a plastic scoop or coring tube), and this is placed in a labelled container for transport to the laboratory. Whirlpak bags and wide-mouth jars (glass or plastic) are

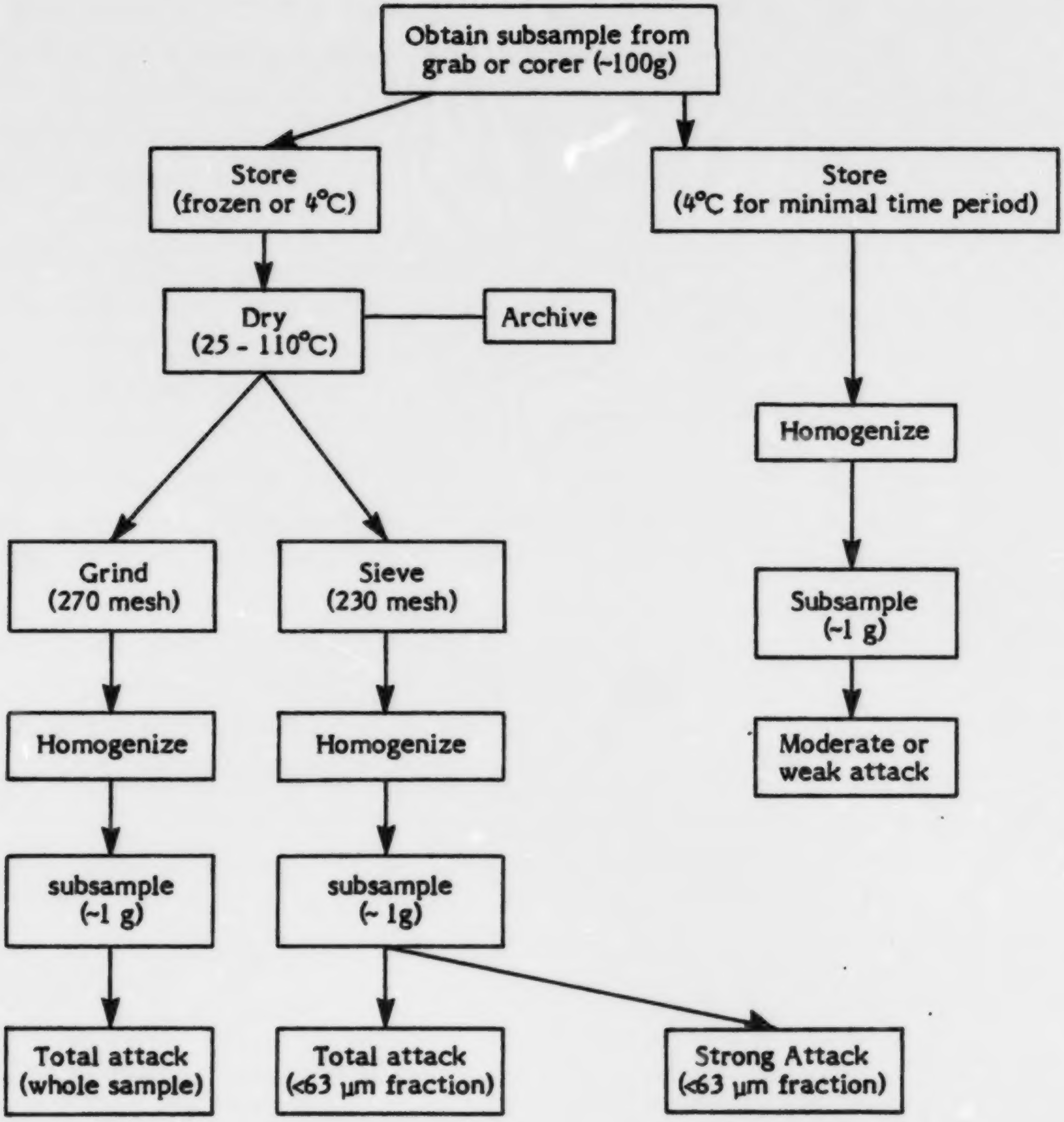

Obtain subsample from grab or corer (~100g)
Store (frozen or 4°C)
Store (4°C for minimal time period)
Dry (25 - 110°C)
Archive
Homogenize
Grind (270 mesh)
Sieve (230 mesh)
Subsample (~1 g)
Homogenize
Homogenize
Moderate or weak attack
subsample (~1 g)
subsample (~ 1g)
Total attack (whole sample)
Total attack (<63 µm fraction)
Strong Attack (<63 µm fraction)

Figure 6.2. Sample preparation sequence for sediments.

usually employed for temporary storage. These should be clean, but special acid cleaning procedures are probably not necessary. Containers should be tested for potential contamination effects. Direct contact of the wet sediment sample with metal surfaces should be avoided. The samples should be stored frozen or at a sufficiently low temperature (4°C) to limit biological activity. For long-term storage (archiving), samples should be stored dry. It is recommended that sediment samples be dried in the laboratory before they are analyzed. Dried sediments are easier to sieve, grind and homogenize than are wet sediments. Metal concentrations are usually expressed on a dry weight basis, so drying of a separate subsample is necessary if wet sediments are to be analyzed. For determining most metals, drying at temperatures from 25-100°C, can be recommended. Freeze drying may be preferred to air or oven drying because it results in a powdery material; air or oven drying of clay sediments results in hard aggregates. **Mercury is sensitive to drying; oven drying should therefore be limited to a temperature of 40°C.** Air drying and freeze drying can also be recommended for Hg.

In order to obtain a representative small subsample (~1 g) for digestion, the sample should be reasonably homogeneous. Grinding of dry sediments to a uniform particle size facilitates homogenization as does screening to remove particles larger than say 63 µm. Homogenization of wet sediments can be effected with a plastic spatula or else by kneading the sample in a plastic bag.

Grinding of sediments also increases the surface area and promotes more rapid dissolution. Grinding is recommended for total attack methods, but is not recommended for strong attack methods because precision and comparability may be degraded. Grinding of dry sediments can be accomplished without contamination using an agate mortar and pestle. Sieving of dry sediments to ca. 230 mesh (63 µm) is recommended for removing the often dominating effect of grain size on trace metal distributions (Forstner and Wittman, 1979; Salomons and Forstner 1984). Sieving is obviously not appropriate when an analysis of whole sediments is required, but is particularly appropriate when a strong attack is used to survey anthropogenic inputs. Nylon sieves are recommended to avoid contamination. When moderate or weak attack methods are used, or when particular species are determined, it is recommended that whole, wet sediments are extracted as soon as possible after collection; drying, sieving and grinding tend to introduce undesirable artifacts.

6.2.3 Sample Preparation Methods for Tissues

Methods for preparing biological samples for trace metal determination must ensure that the sample is representative of the tissue or organism that is being studied and that its composition has not been altered by handling or storage. After the organisms have been retrieved, they should be washed with clean seawater to remove sediments and adventitious contamination. Depuration of clams and mussels is optional; it is recommended whenever there is a concern that food or sediments inside the gut may vitiate the results. The organisms can be stored frozen in plastic bags or glass or plastic jars. These should be very clean but acid purification is probably not necessary. To avoid necrochemical artifacts the organ or tissue that is to be analyzed should be removed immediately after sampling. If that is not possible the whole organism should be analyzed (minus the shell in the case of bivalves). Freeze drying of the tissue sample is recommended for long term storage.

As all tissue samples will be heterogeneous, they should be homogenized before subsampling. This can be accomplished by mincing, chopping or blending the wet sample, either by hand or by machine. Care should be taken to choose the right implement at this stage as some may contaminate the sample with the metals being analyzed. For example, stainless steel homogenizers (such as Polytron or UltraTurrax) may be safe for arsenic and mercury, but are definitely not safe for Fe, Cu, Ni and Cr. Alternatively, the tissue sample can be homogenized after drying by grinding in an agate mortar and pestle. Drying also facilitates long term storage. For elements other than mercury, oven drying at up to 100°C is recommended. When mercury or methylmercury is being determined, only freeze drying can be recommended. To be entirely safe, non-metallic tools and labware should be used throughout. After homogenization and subsampling, the wet or dry tissue sample is digested by wet or dry ashing techniques. If it is digested wet, a separate subsample should be dried to determine the moisture content, since concentrations are usually expressed on a dry weight basis.

6.3 Instrumental Methods

6.3.1 Factors Affecting the Choice of Instrument

Usually the first decision to make is which instrument to use. The choice depends on which are sensitive enough to determine the metal concentrations in the samples. The final metal concentrations in the solutions presented to the instrument should be within the linear operating range of the instrument. Whether to employ a preconcentration step depends on whether the metal concentrations after digestion are below the limits of quantification and whether other elements in the sample matrix interfere strongly with the elements being determined. Thus the decision of which instrument to use, and whether to employ preconcentration interact. The kind of digestion that is appropriate also sometimes depends on the choice of instrument. Flame AAS, for example, can tolerate more dissolved solids and viscosity than can ICP and GF-AAS. Hydrofluoric acid is incompatible with the nebulizer systems commonly used with AAS and ICP. Sulfuric acid can produce an undesirable amount of background absorption with GF-AAS.

Instrumental sensitivity decreases in the order, GF-AAS $\gg$ ICP > F-AAS, whereas the metal concentrations generally increase in the order, interstitial water $\ll$ tissues < sediments. This relationship explains why interstitial waters are analysed almost exclusively by GF-AAS after preconcentration and why sediments are usually analysed by F-AAS or ICP. GF-AAS is too sensitive to determine most elements in sediments and is therefore usually reserved for those elements for which F-AAS and ICP are not sensitive enough.

To estimate whether the final solution concentrations will be within the operating ranges of the instruments requires some assumptions. The sample concentrations must first be assumed. These are given in Tables III-2.3 (interstitial waters), III-2.8 (sediments) and III-2.17 (biota) of Volume I. Sample concentrations, together with the dilution factor (for the digestion) and the pre-concentration factor, determine the final solution concentrations. Typical values for these factors are assumed; sometimes different combinations are explored. It is assumed that the method limit of quantification, L.O.Q., is equal to ten times the instrumental limit of detection, L.O.D., which are given in Table III-2.1 of Volume I. The upper limit of the linear operating range is assumed equal to 10^3 x L.O.D. for flame and GF-AAS and 10^5 x L.O.D. for ICP.

The procedure used for estimating whether the final solution concentrations will be within the linear operating range of the instrument consists of:

1) calculating the final solution concentration range from the original sample concentration range and the typical dilution and preconcentration factors, and,

2) comparing this with the linear operating range of each instrument for each metal.

The results are then presented in a table in which the metals are divided into four categories:

1) **too low** - sample concentration range is completely below the operating range of the instrument;

2) **marginal** - sample concentration range is partly below the lower limit of the operating range;

3) **within** - sample concentration range is within the operating range;

4) **too high** - sample concentration range is partly or completely above the upper limit of the operating range.

Finally, the results are checked against the published method detection limits or limits of determination quoted in Volume 1. Method detection limits are not always defined in the same way. The exact definitions are sometimes but not always given in the papers describing the methods. In general, the method detection limits correspond roughly to the criterion used here, i.e. 10 x L.O.D. (instrumental), and the two sets of results are consistent.

After determining which instruments are suitable from the standpoint of sensitivity, other considerations come into play in making a choice of instrumental method. Accuracy and precision can be assessed, since specific information on these parameters is usually given in method reports. Accuracy equals absence of specific interferences and is usually proven by analysis of standard reference materials (SRM's). Of the three instrumental techniques, graphite furnace is generally the most subject to interference and, after automatic corrections for spectral interference, ICP is generally the least. Since different kinds of interference affect F-AAS and ICP, the two instruments could be used synergistically to check the accuracy of instrumental quantification. The overall precision of the determination is ultimately limited by the instrumental precision. Usually however, the overall precision does not approach this limit very closely. Also, the instrumental precisions are not widely different, so it is assumed that the instruments are equivalent in this respect.

Although specific cost information is not usually given in method reports, the factors affecting cost can be estimated. Instrumental costs decrease in the order ICP > GF-AAS > F-AAS. Sample throughput for F-AAS is greater than for GF-AAS. ICP has a potentially much greater throughout than F-AAS since a large number (20-30) of elements can be determined simultaneously. Whether a preconcentration step is necessary will affect the analysis time. In terms of operational difficulty, ICP is difficult to learn but easy to operate routinely. Flame AAS is easy to learn and to operate. GF-AAS is moderately difficult to learn and to operate. In terms of availability, F-AAS > GF-AAS = ICP. Taking all factors into consideration, the cost per element is expected to be somewhat greater for GF-AAS than for F-AAS. Depending on the number of elements to be determined, ICP can be more expensive than F-AAS (for fewer than about 3-5 elements) or cheaper (for more than that number). Commercial laboratories usually charge a fixed amount for an ICP determination, which quantifies 20-30 elements. Therefore, the cost per element needed is inversely proportional to the number of elements, and ICP would be very much cheaper than AAS if values for 20 - 30 elements were needed. It is unusual however, for a multielement method to give accurate values for that many elements.

It may be advantageous to use two or more instruments for determining a particular combination of metals in the same solutions. For example, rather than use GF-AAS for Cr, Cu, Cd, Ni and Pb in sediment solutions, it would be cheaper and probably more accurate to use F-AAS for Cr, Cu and Ni, and to use GF-AAS for Pb and Cd, which require GF sensitivity. In most cases, the same sample solutions can be quantified by different instrumental techniques; when a large number of elements must be determined, ICP has a big cost advantage over F-AAS, and would therefore be preferred, all other things being equal.

6.3.2 Interstitial Waters

6.3.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

The analysis of seawater and interstitial water for Group I metals places high demands on the sensitivity of the instrument. There is little published data on trace metal concentrations in interstitial waters. In assessing the suitability of different instrumental techniques, it has been assumed that they vary from normal seawater levels to those that are hundreds of times greater. Theoretical L.O.Q.'s were not calculated. At such low concentrations they may be more dependent on the blank than on the instrumental sensitivity. Instead, the method L.O.D.'s or procedural blanks quoted in Volume 1 were directly compared with the normal seawater concentration ranges given in Table III-2.3, Volume I. From these comparisions, it is clear that sensitivity decreased in the order: GF-AAS (with preconcentration) > GF-AAS (without preconcentration) $\simeq$ ICP (with preconcentration). While GF-AAS with preconcentration is sufficiently sensitive to determine most of the metals at the lower ends of their concentration ranges, the latter two techniques are not. Flame AAS with preconcentration is even poorer, and for this reason it is vary rarely used.

The method L.O.D.'s depend on the sensitivity of the instrument, the preconcentration factor and the magnitude of the blank. To obtain a very high preconcentation factor, e.g. 500, it is necessary to extract a large volume of seawater, 500 - 1,000 mL. A 500 - 1,000 mL sample of interstitial water is impractical however, since interstitial water samplers (pressure squeezers and centrifuge) generally yield volumes in the range of 10 - 100 ml. It may be possible to reduce the volume needed for ICP quantification by employing a microsampling technique similar to the one described by Uchida et al. (1981). The successful application of this technique to interstitial waters would be a significant advance. On the other hand, preconcentration factors of 50 -200 are sufficient for GF-AAS and a final sample volume of 0.5 - 1 ml is adequate for doing a suite of five trace metals, which is a typical yield for a preconcentration. Thus the superior absolute sensitivity of GF-AAS is a very significant advantage in the analysis of interstitial waters.

RECOMMENDATION

On the basis of sensitivity, GF-AAS is the most appropriate choice. Although it may be possible to get by with a direct GF method for some metals in some samples, preconcentration will probably be necessary in most instances. The preconcentration/GF-AAS methods can be scaled down to the smaller sample volumes typically available for interstitial waters. The preconcentration/ICP methods will probably be generally less useful due to the larger sample volumes required, although the ability to analyse a large number of elements simultaneously is a compensating advantage.

Assuming that the instrumental costs would be similar for the analysis of digested sediment or tissue samples, then there would be a significant cost advantage for multielement analysis utilizing ICP. However, adapting an ICP method to the smaller sample volumes available may entail an unacceptable sacrifice to the detection limits. Therefore ICP will not always be preferred when more than 3-5 elements are to be determined.

Direct GF-AAS methods may be somewhat cheaper than preconcentration/GF-AAS methods, but they are not sensitive enough to determine the lowest expected concentrations. Direct GF-AAS methods also suffer from the following disadvantages: they are more prone to interferences; they are equipment dependent, and they are more difficult to apply. The preconcentration methods do not have these disadvantages. Solvent extraction, the most popular technique, is fairly rapid, and it effectively removes the seawater matrix, making the instrumental determination much easier and faster. Starting with a volume of 10 -100 ml, the final volume is quite adequate and the final concentrations are nearly optimum for the graphite furnace. When three or more metals are preconcentrated at once, the total analysis time would probably not be greater than when using direct GF methods.

A possible disadvantage of preconcentration methods is incomplete recovery of metals bound to colloidal organic matter. This problem can be circumvented by employing a sample digestion method, as described in section 6.5.2.

6.3.2.2 Instrumental Methods for Arsenic in Interstitial Waters

For the determination of As in interstitial waters, arsine generation is the only technique which is sensitive enough to measure As in seawater and interstitial water. Although arsine can be quantified by gas chromatography (with electron capture detection), DC plasma atomic emission spectrometry or ICP-AES, the most popular and sensitive detection system is a quartz cuvette burner in the light path of an AAS. The use of a cold trap to condense arsine and organoarsine compounds purged from a sample combined with electrical heating to selectively volatilize the compounds increases sensitivity and enables the determination of four arsenic species in sea water, arsenate, arsenite, monomethylarsonic acid and dimethylarsenic acid. The alternatives to hydride generation AAS is graphite furnace AAS after preconcentration by ion exchange or solvent extraction. These techniques are less sensitive, more time consuming and more restricted in the species that can be determined.

The cold trap/HG-AAS method of Andreae (1977, 1983) has the best combination of desirable features for determining arsenic species in seawater and interstitial waters. It has been extensively applied and meaningful results have been obtained. The detection limits are excellent (0.3 ng/L), the analysis time is 30 minutes and only 10-100 mL of sample is required. Interferences are minimal and contamination is not as big a problem as with some other elements, e.g., mercury. Samples can be preserved by quick-freezing them without altering the species ratios. The apparatus is specialized but not difficult or expensive to fabricate. Commercial systems are also available (e.g., from Varian and Perkin-Elmer).

6.3.2.3 Instrumental Methods for Hg in Interstitial Waters

a) Total Mercury

The mercury vapour technique, in which mercuric ions are reduced to elemental mercury and partitioned into the gas phase, must be used to determine Hg in seawater and interstitial waters. A quartz cuvette in the light path of an atomic absorption spectrometer is the most common detection system; atomic fluorescence and DC plasma emission spectrometry are considerably rarer. The other common preconcentration instrumental techniques do not have sufficient sensitivity. The sensitivity of the mercury vapour technique is considerably improved and potential interferences are reduced when a gold trap is placed between the sample reduction flask and the optical cell of the AAS. A gold trap consists of a quartz tube containing gold foil or gold-coated glass beads; gold amalgamates the Hg vapour swept from the reduction flask. When the trap is heated by a coil of resistance wire, Hg is quantitatively released and transferred by a stream of argon or nitrogen gas to the detector.

The method of Olafsson (1983) typifies the gold trap CV-AAS technique of determining mercury in sea water. The lower limit of quantitative determination is about 2.5 ng/L. The two stage gold trap CV-AAS method of Bloom and Crecelius (1983) has the ability to determine Hg in sea water down to 0.1 ng/L. The accuracy and precision was demonstrated by a collaborative study and by analysis of SRM's. Advances in the use of extremely clean reagents and special sample handling techniques are partly responsible for achieving the extremely low detection limit. **The methods of Olafsson (1983) and of Bloom and Crecelius (1983) are well proven and should not present any special difficulty in their application to interstitial water.**

The automated CV-AAS method of Goulden and Anthony (1980) presents an interesting alternative for measuring mercury and its species in interstitial waters.

Through a selective reduction scheme, the method is able to determine alkyl, aryl and inorganic mercury. The detection limit, 1 ng/L, is achieved through efficient partitioning of elemental Hg into the gaseous phase. The advantages of requiring less attention and having greater throughput and precision than the manual methods are offset somewhat by the greater initial cost and difficulty of setting up the apparatus.

b) Methylmercury

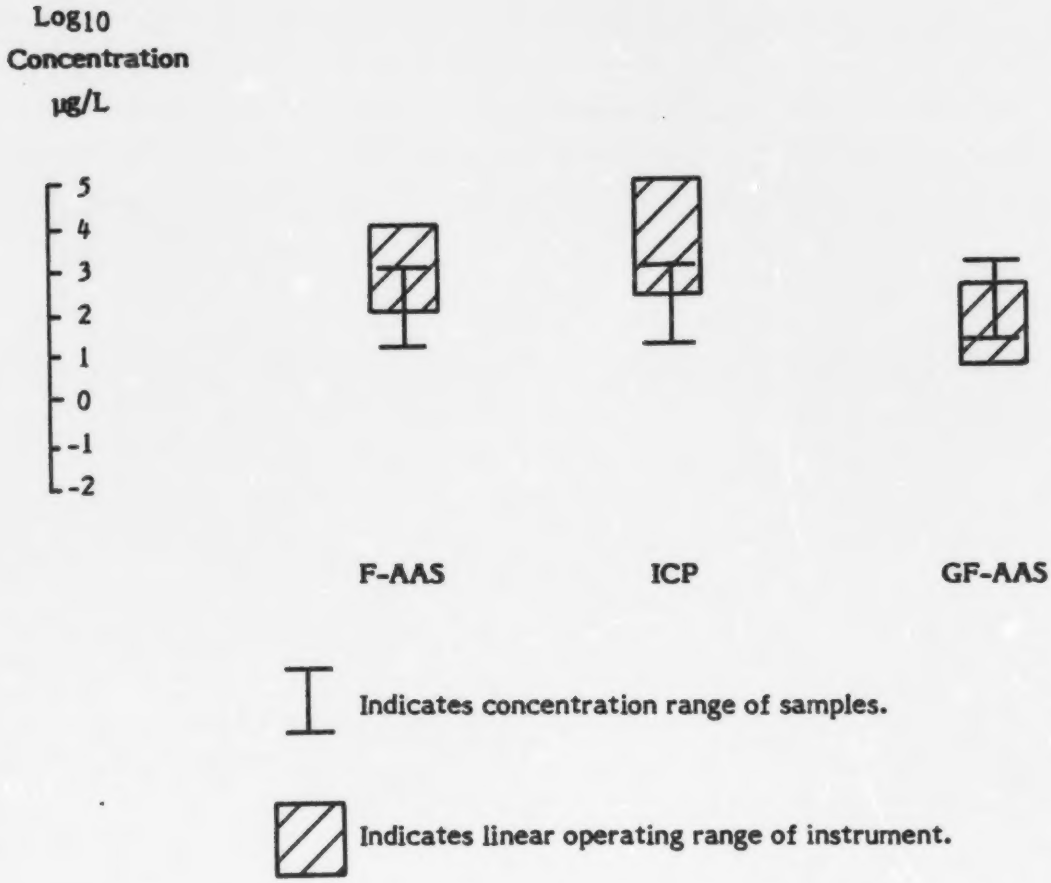
No thoroughly satisfactory methods have been found for determining methylmercury in interstitial water. Goulden and Anthony's (1980) method will determine methylmercury by difference to 5 ng/L. The sensitivity of this method could be extended by combining the selective reduction technique with the manual gold trap CV-AAS technique. A more selective direct method could presumably be derived from the benzene extraction/GC-EC methods for biota described in Section 6.4.4.2.

6.3.3 Instrumental Methods for Sediments and Biota

6.3.3.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

a) Sediments

In determining which instruments have sufficient, but not too much, sensitivity for determining metals in Arctic sediments, the concentration ranges of Table III-2.8, Volume I were used. The dilution factors arising from digestion were taken to be 100 for F-AAS, GF-AAS and ICP. The general procedure has been described in section 6.3.1. To illustrate, results for Pb are given below.



The results show that F-AAS and ICP would not be effective choices. Lead can be quantified by GF-AAS at 100 fold dilution, however. The results for Group I elements plus As and Hg are summarized in the table below.

	F-AAS	ICP	GF-AAS
Too low	As, Be, Hg	Cd, Hg, As	Hg
Marginal	Cd, Pb	Pb	
Within	Ba, Cr, Cu, Ni, V	Ba, Be, Cr, Cu, Ni, V, Zn	As, Be, Cd
Too High	Zn		Ba, Cr, Cu, Ni, Pb, V, Zn

It can be concluded that GF-AAS is indispensable for a few elements, but generally too sensitive for most. F-AAS and ICP are generally the most useful instruments in that they both can quantify the majority of metals. GF-AAS can be used to complement either F-AAS or ICP. These results are believed to be fairly general but some of the details might change if different sample concentrations, instrumental detection limits or preconcentration factors are used. Therefore, it is recommended that the exercise be repeated using more appropriate data when they are available.

The choice between ICP and F-AAS is not restricted by sensitivity. The cost of analysis by ICP is likely to be much lower than by F-AAS when a large number of elements are determined. In commercial laboratories, the determination of 20-30 elements by ICP costs about the same as the determination of 3-5 elements by F-AAS.

Typically, GF-AAS costs a few dollars more per element than F-AAS. Where special techniques are used, such as the L'Vov platform, the price difference will be greater. These conclusions are based on the prices charged by four commercial laboratories shown in Table III - 2.12, Volume I. From the evidence presented in Volume 1, the overall accuracy and precision of the determination of metals in sediments by ICP is at least as good as that accomplished by F-AAS. The instrumental precision of F-AAS is slightly better than GF-AAS, although the overall method precision is not only limited by the instrumental precision. In comparing flame and graphite furnace for accuracy in the analysis of sediment digests, interferences are generally more likely to be encountered with the graphite furnace, but for the methods in question, the interferences have been mitigated by using standard additions or matrix modifiers. Matrix modifiers are also used in the flame methods. In the case of ICP, interferences are mainly spectral in character and are effectively corrected by spectral analysis and calculation. Since method accuracy is normally proven by analysis of reference materials, it can be concluded that serious interferences are absent. The instruments therefore appear to be on an equal footing with respect to accuracy.

RECOMMENDATION

F-AAS and ICP are the methods of choice to determine most metals in sediments. GF-AAS will probably be needed only for As, Cd, Pb and Be. Arsenic can be done with better accuracy by hydride generation AAS. Mercury must be done by cold vapour AAS. ICP is preferred to F-AAS when more than 3-5 elements are determined, because ICP is less expensive than F-AAS and just as precise and accurate. An ICP determination gives the concentration of 20-30 elements, so it is sometimes used to screen samples for possible anomalies. Flame AAS is cheaper when less than 3-5 elements are to be determined. Flame AAS and ICP are complementary in the sense that they have different kinds of interferences.

The instrumental methods of McLaren et al. (1981) Floyd et al. (1980) and McQuaker et al. (1979) can be recommended for ICP. The methods employ different instruments, sequential and simultaneous spectrometers, which have different characteristics and are operated differently. The methods describe how to correct for spectral interferences, which are individual for the instruments. It is thus necessary to perform a spectral analysis during method development. The effectiveness of correction must then be checked by analysis of SRM's.

The instrumental methods of Loring and Rantala (1977) and Agemian and Chau (1975a) can be recommended for F-AAS. These methods use direct comparison of sample solutions with pure (mixed) standards made in the same acid matrix. Matrix modifiers are required for certain elements, and the two methods differ in this respect. Loring and Rantala's method has been validated by analysis of SRM's, whereas Agemian and Chau's has not. As suggested by Loring and Rantala, dilution factors (arising from digestion) of 100 - 2,000 can be applied as necessary.

The instrumental methods of Sturgeon et al. (1982) are recommended for determining Be, Cd, and Pb by GF-AAS. (The paper also gives methods for Cu, Ni, Co and Cr which probably do not need to be determined by GF-AAS.) The stabilized temperature plaform furnace (L'Vov platform) reduces matrix interference effects, eliminating the need for standard additions. The matrix modifiers and graphite furnace conditions given by Slavin et al. (1983) are also recommended. For many of the less volatile elements (including Ba, V, Cu and Ni) the L'Vov platform gives reduced sensitivity compared to a normal pyrolytically coated graphite tube. For these elements, the recommendations of Slavin et al.(1983) should be followed. The pyrolytic tube/matrix modifier/standard addition methods of Caravajal et al.(1983) and the methods of Sakata and Shimoda (1982) and Rantala and Loring (1980) for Cd also contain relevant information on GF techniques.

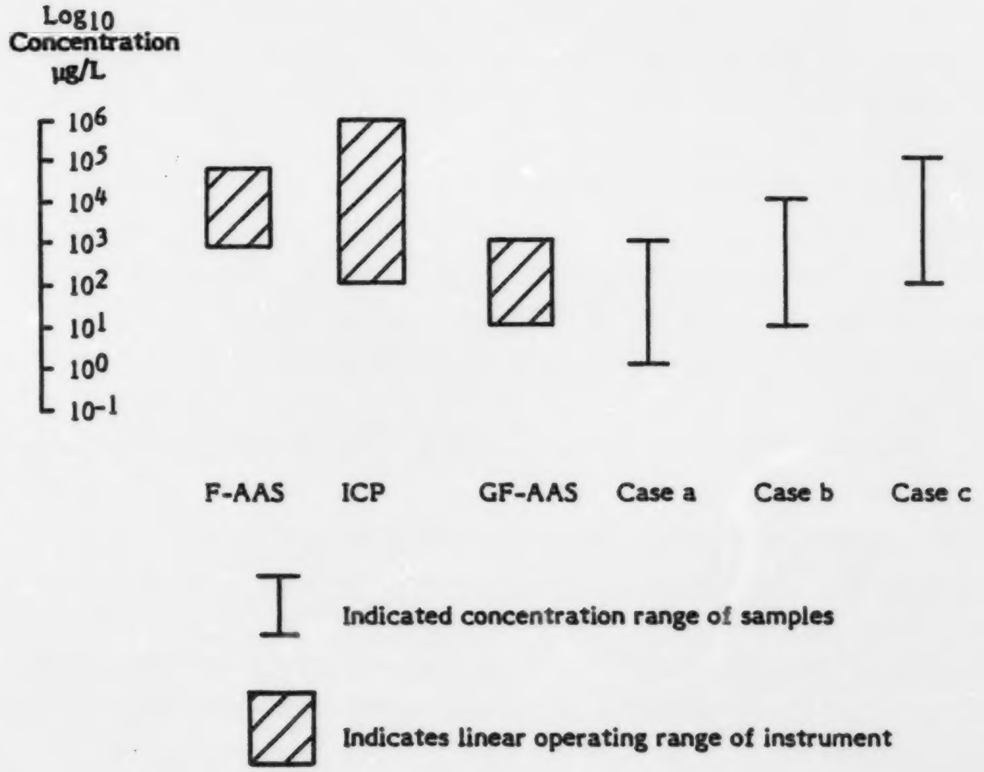
b) Biota

The general procedure for determining which instrumental techniques have sufficient, but not too much, sensitivity is described in section 6.3.1. In the analysis of tissues, it is assumed that digested tissue solutions can be analyzed either directly or after preconcentration. It is assumed that digestion will produce a 100 fold dilution of the sample (final solution weight/original sample dry weight) and that preconcentration factors of 10 and 100 are possible. Thus, nine different combinations, 3 instruments and 3 solution concentrations, are evaluated. The solvent enhancement effect for aspiration of a combustible organic solvent into the flame of an AAS is not taken into account. The tissue concentration ranges the metals (μg/g, dry weight derived from Table III - 2.17 Volume I) are assumed to be:

As	.1 - 100	Hg	.002 - 10	Zn	2 - 2000
Cd	.01 - 10	Ni	.02 - 20		
Cr	.1 - 100	Pb	.03 - 30		
Cu	.1 - 100	V	.05 - 50		

These are generally less than the sediment concentrations, by factors of 10 -100.

Arsenic is taken to illustrate the procedure. The concentration range for As in tissues is 0.1 -100 μg/g. For digestion without preconcentration (case a), the final solution concentrations are 1-1000 μg/L. For digestion with 10x preconcentration (case b), they are 10-10,000 μg/L, and for digestion with 100x preconcentration (case c), they are 100-100,000 μg/L. The diagram below shows the instrumental operating ranges and final solution concentration ranges side by side.



It can be seen that As can be done by GF-AAS with a 10x preconcentration or by ICP with a 100x preconcentration.

The results for other metals are tabulated below.

	F-AAS		ICP		GF-AAS	
	Case b	Case c	Case b	Case c	Case a	Case b
Too low	Hg	Hg	Hg	Hg	Hg	
Marginal	As,Cd,Ni, Pb,V	As,Pb	As,Cd, Ni,Pb	Pb	As,V, Ni,Pb	Hg
Within	Cu,Cr	Cd,Cr, Ni,V	Cr,Cu, V,Zn	As,Cd,Cr, Cu,Ni,V,Zn	Cu,Cr,Cd	As,Cd,Cr, Cu,Ni,Pb,V
Too high	Zn	Zn			Zn	Zn

From these results it can be concluded that if flame or ICP is to be used, a 100 fold preconcentration (case c) is necessary for most elements. If GF-AAS is to be used, then a 10x preconcentration (case b) is sufficient for most elements. A number of elements can be done by GF-AAS without preconcentration. The results for F-AAS may be biased somewhat due to neglect of the solvent enhancement effect, which can lower detection limits by a factor of 2 or more. These conclusions are generally consistent with the L.O.D.'s given for specific methods in Tables III-2.16 and 2.18 of Volume I, however.

The choice of flame, graphite furnace or ICP for the analysis of tissues is not restricted by sensitivity when preconcentration is allowed. Although it increases the cost of the analysis and adds to the difficulty, a preconcentration step is often unavoidable, since the graphite furnace is not sensitive enough to determine many of the elements directly. Preconcentration has the compensating advantage of reducing interference from Na, K, Ca and Mg, etc., making the analysis potentially more accurate. When a preconcentration step is required, it would be advantageous to use flame or ICP whenever possible. However, when the quantity of tissue that is available for analysis is very limited, the graphite furnace would be preferred since it requires considerably less (only 10-100 mg dry weight) tissue. Comparing ICP and F-AAS, ICP would be preferred when more than 3-5 elements are to be determined. This number of elements is a typical yield for a preconcentration method. Flame and ICP can be used together as an accuracy check on the instrumental determination.

RECOMMENDATIONS

The procedures given by Evans et al. (1980) and Evans et al. (1978) are recommended for F-AAS. These references contain useful information on the optimization of instrumental conditions as well as the accuracy, precision and detection limits obtainable. These procedures apply to the analysis of digested and preconcentrated tissue solutions. Some relevant information may also be contained in the papers by Loring and Rantala (1977) and Agemian and Chau (1975a), which describe the more difficult problem of the analysis of digested sediment solutions.

For the analysis of tissue preconcentrate by ICP, the procedures of Evans and Dellar (1982) are recommended. There is much useful information on instrumental conditions, calibration, interferences, and obtainable performance in this report. Jones et al. (1982) give little information on instrumental technqiues. For the analysis of tissue digestate by ICP, the information developed by McQuaker et al. (1979) is relevant. The information given by McLaren et al. (1981) and Floyd et al. (1980) on analysis of sediment digestate solutions may also be useful in developing an instrumental method for tissues. Spectral line interferences are more important in digestate than in preconcentrate solutions.

For the analysis of tissue digestate by GF-AAS, the stabilized temperature platform furnace (STPF) technique (Sturgeon et al. (1982), Fernandez et al. (1981) and Hinderberger et al. (1981)) is recommended. This technique applies especially to the more volatile elements such as Cd, Pb and As. For these metals, more accurate results will be obtained for less effort than atomization "off the wall" of a conventional graphite tube. Matrix modifiers are sometimes used in conjunction with the STPF; Slavin et al. (1983) gives graphite furnace conditions and matrix modifiers for a variety of elements. For the more refractory elements, e.g., Ba, Be, V, Cu and Ni, the STPF technique does not appear to offer any advantages. Borg et al. (1981) give instrumental settings for conventional graphite furnace determination of Cu, Pb and Cd in tissue digestate solutions. For determining Be in biological tissues, the preconcentration/GF-AAS method of Hurlbut (1978) is recommended.

6.3.3.2 Instrumental Methods for Arsenic in Sediments and Biota

From the standpoint of sensitivity, the hydride generation methods are all adequate for determining arsenic in sediments and tissues. Both AAS and ICP based hydride methods are available. GF-AAS has sufficient sensitivity for determining arsenic in sediments without preconcentration, whereas a 10 fold preconcentration is necessary and sufficient for determining the lower levels of As found in tissues. ICP is almost sensitive enough to determine As in sediments, but not in tissues.

In terms of accuracy and precision, the hydride generation methods are better than the direct graphite furnace methods. After preconcentration by solvent extraction, GF-AAS will be much less subject to interferences. For sediments, direct GF-AAS is cheapest, especially when other (Group I) elements are determined on the same sample solutions. In commercial laboratories, HG-AAS or HG-ICP is about 2-3 times more expensive than direct GF-AAS, unless only arsenic is determined in which case the costs are about equal (Table III-2.14, Volume I). The cost of determining arsenic in tissues by GF after preconcentration would be similar to that for HG-AAS. HG-ICP has the advantage that several other hydride forming elements can be determined with arsenic for the same cost as determining arsenic alone by HG-AAS. From a brief survey of commercial laboratories in the Vancouver - Victoria area, it appears that instrumental set-ups for determining arsenic by hydride generation-AAS or ICP are common. The continuous hydride generation methods are superior in throughput and ease of operation, because they are automated. Though slightly less sensitive, the continuous methods should have a significant cost advantage whenever large numbers of samples are run routinely.

References to recommended instrumental methods are given in Table 6.1.

6.3.3.3 Instrumental Methods for Hg in Sediments and Biota

a) Total Mercury

CV-AAS techniques must be used for measuring mercury in sediments and biota. Flame AAS, GF-AAS and ICP have inadequate sensitivity. The automated or continuous CV-AAS methods have adequate detection limits for most sediment and tissue samples, but not for those having the lowest expected concentrations. The manual methods, which have detection limits that are about an order of magnitude lower, are adequate for the lowest concentrations. The automated methods will be preferred when sample concentrations are high enough and when large numbers of samples need to be analyzed, due to their higher throughput and slightly better precision. Automated instruments also require less attention to operate, but are more expensive and more difficult to set up.

Table 6.1

Instrumental Methods for Arsenic in Sediments and Biota

HG-AAS	HG-ICP	GF-AAS
a) Sediments		
continuous: Agemian & Bedek (1980), Vijan et al. (1976) manual: Rubeska and Hlavinkova (1979)	continuous: deOlievera et al. (1983), Pahlavanpour et al. (1980), Goulden et al. (1981)	Caravajal et al. (1983) - direct GF-AAS method - matrix modifier, standard additions used Slavin et al. (1983) - STPF, Zeeman background corrector used
b) Biota		
continuous: Agemian and Thomson (1980), Arafat and Glooschenko (1981) manual: Evans et al. (1979), Kuldvere (1980)	continuous: Goulden et al. (1981) deOlievera et al. (1983)	Thiex et al. (1980) - solvent extraction/ GF/AAS method

The recommended instrumental methods are given in Table 6.2. The manual techniques exist in a variety of configurations (see Section III-2.3.3 - Volume I for descriptions), all of which have approximately the same limit of detection. The stationary method of Tong and Leow (1980) is somewhat slower than the other manual methods, but has the advantage of simplicity. The Perkin Elmer MHS-1 mercury/hydride system (Kuldvere and Andreassen, 1979) and the Varian model 65 vapour generation accessory (MacPherson et al, 1982) are easily obtained and seem to perform as well as the other manual methods.

b) Methylmercury

There are three instrumental techniques for determining methylmercury in fish tissue - gas chromatography with electron capture detection, CV-AAS and GF-AAS. Of the three, GC-EC is the most commonly used. The detection limits of the GC-EC and CV-AAS methods are similar (0.05 - 0.1 µg/g) while that of GF-AAS is higher. The GC-EC methods are superior to the others in selectivity, since the gas chromatograph is capable of resolving the organomercurial compounds while the other instruments cannot differentiate them. Assuming that the costs are approximately equal, GC-EC is preferred since it has better selectivity and equal or better sensitivity. CV-AAS would be preferred if GC-EC were not available, since it is more sensitive than GF-AAS.

Before the above mentioned instrumental techniques can be used to quantify methylmercury it is necessary to employ a method to separate it from the sample. Preconcentration methods for methylmercury in tissues are discussed in Section 6.4.4.2. References for the instrumental methods are given with the recommendations for preconcentration methods.

6.4 Preconcentration Methods

6.4.1 General Considerations

As mentioned previously, preconcentration procedures are almost always applied to sea water, often applied to tissues and seldom applied to sediments. Sea water has extremely low concentrations of trace metals relative to major and minor cations, i.e. Na, K, Ca and Mg. Tissue digests have a composition of major and minor cations that is similar to seawater, but the trace metals are relatively more

Table 6.2

Instrumental Methods for Mercury in Sediments and Biota

	Automated CV-AAS	Non-automated CV-AAS
a) Sediments	Agemian and Chau (1975b) - manifold system - 20 samples/hour sampling rate	MacPherson *et al*. (1982) - equilibrium partition method - Varian model 65 vapour generation accessory Ure and Shand (1974) - equilibrium partition method
b) Biota	Agemian and Chau (1975b) Agemian and Cheam (1978) - manifold system - 20 samples/hour sampling rate	Kuldvere and Andreasson (1979) - Perkin Elmer MHS-1 mercury/hydride system Chapman and Dale (1978, 1982) - equilibrium partition method - transfer of Hg vapour by displacement with water Tong and Leow (1980) - stationary CV-AAS method

concentrated in tissues. Sediment digests have a rather different composition of major and minor elements (e.g. Fe, Al and Si are much higher), and analyte concentrations are higher in sediments than in tissues. The high concentrations of Fe and Al can pose problems in applying preconcentration methods to sediments.

Preconcentration methods are sometimes needed:

1) **to bring the analytes into the operating range of the instrument and**

2) **to remove interferences arising from the matrix of major and minor elements.**

The use of a preconcentration step increases the cost of the analysis and should not be used unless it can be justified. How sample concentration and instrumental detection limits can be used to decide which instruments are appropriate for an analysis has been described in Section 6.3.1. Which instruments are appropriate also depends on whether a preconcentration method is available. The decisions to use particular instrumental and preconcentration methods thus interact.

Interferences arising from the major and minor elements are very common. Although they can sometimes be mediated by special instrumental techniques, such as matrix modifiers and special temperature programs on the graphite furnace, using a preconcentration method is usually the most effective way to deal with the problem, because the interfering elements are almost entirely removed without affecting the concentration of analytes. Interferences are common but are very hard to anticipate since they depend on the particular combination of analyte and interfering elements and are caused by a variety of physical and chemical mechanisms. In general, the potential for interference diminishes in the order: GF-AAS > F-AAS > ICP. In the case of ICP, interferences are mainly of the spectral type, for which exact corrections can be made.

Preconcentration methods are generally indifferent to the (total) digestion method used since the interfering cations are affected in the same way by the different digestions and the anions introduced by the acids usually do not interfere with the preconcentration methods. Similarly, since the interfering alkali and alkaline earth metals are almost completely removed from the solution containing the analytes, instrumental methods are generally not sensitive to the preconcentration method used. Thus preconcentration methods can generally be considered to be interchangeable. There is no essential difference between the methods that are used for GF-AAS and ICP, for example.

Concentration factors resulting from preconcentration methods are variable; they may be as low as 5 or as high as 500. The concentration factor can be adjusted to suit the needs of the particular analysis situation, e.g., it may be adjusted to enable a particular limit of detection to be achieved. An upper limit for the concentration factor exists for every preconcentration technique. As this limit is approached the separation becomes less quantitative. The limits for concentration factors are not usually given in methods reports and some experience is required to estimate them. The limiting concentration factors can be obtained by experiment, and thus concentration factors can be regarded as a variable under the control of the experienced analyst.

Most preconcentration methods are multielement methods. Up to nine metals can be co-extracted from sea water using dithiocarbamate chelating agents. There is a substantial increase in efficiency when a single preconcentration suffices to do a number of elements, since the preconcentration step can consume a large portion of the analysis time.

6.4.2 Preconcentration Methods for Interstitial Waters

6.4.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

As previously mentioned, preconcentration methods are usually necessary for measuring trace metals in sea water and interstitial water. Preconcentration serves two important purposes: removal of the interfering alkali and alkaline earth metals and raising the concentration of the analytes to be within the operating range of the instrument. **The principal techniques used for sea water are: 1) solvent extraction, 2) chelating ion exchange separation and, 3) coprecipitation.**

Of these solvent extraction is certainly the most popular. Solvent extraction offers the following advantages: broad scope, speed, simplicity, high efficiency, high concentration factors and low and reproducible blanks. Most of the lowest detection limit methods combine GF-AAS with solvent extraction preconcentration. A two-fold solvent extraction in which the metals are first extracted into an organic solvent and later back-extracted into nitric acid is preferred, because it produces higher extraction efficiency, greater concentration and the final extract is stable against concentration change with time. In addition, dilute nitric acid is more compatible with GF-AAS and ICP, than are organic solvents.

New chelating ion exchange methods have recently been developed which employ a variety of different chelating functional groups. The chelating ion exchange techniques potentially offer the same advantages as the two-fold solvent extraction techniques, but being newer they are less widely used. Also, some of the new chelating ion exchange resins are not commercially available and must be prepared in the laboratory. The older Chelex 100 technique is still used despite certain drawbacks, and Chelex based methods have recently been improved. Coprecipitation is less important than the other two techniques. It is apparently less developed and fewer multi-element methods are available. Some important single element methods employ coprecipitation, however.

In order to adopt a seawater method for interstitial water analysis, it will usually be necessary to scale it down to use smaller sample volumes. This will usually involve a proportional reduction of all volumes. It may also involve using smaller flasks, pipettes, separatory funnels, extraction columns, etc. Centrifugation may be used instead of filtration. Some useful information can be gleaned from reports (cited in Volume 1) of seawater methods being adapted for interstitial water analysis. Such adaptations may require some trial and error, but in most cases they should be fairly easy to accomplish.

RECOMMENDATIONS

The multielement preconcentration methods have been summarized in Table 6.3. **The two-fold solvent extraction procedures given in the table are recommended for the following reasons.** They are based on a well proven and firmly established technique. The range of metals that can be extracted is as great as chelating ion exchange and greater than Co-APDC coprecipitation. Very high (500 fold) preconcentration factors and very low blanks are possible. The overall performance of the two-fold solvent extraction methods is probably as good or better than any other available method.

The chelating ion exchange method of Sturgeon et al. (1981) offers comparable performance to the two-fold solvent extraction methods, but the chelating ion exchange material (8-hydroxyquinoline chemically bonded to silica gel) must be prepared in the laboratory. Until this material is widely available, the

Table 6.3

Recommended Multielement Preconcentration Methods for Seawater

a) Solvent Extraction Methods

Reference	Metals	Chelating Agents/Extraction Solvents
Danielsson et al. (1978), Magnussen and Westerlund (1981), Danielsson et al. (1982)	Cd, Co, Cu, Fe, Ni, Pb, Zn	APDC + DDDC/Freon TF;Nitric Acid
Bruland et al. (1979)	Cd, Cu, Ni, Zn	APDC + DDDC/Chloroform; Nitric Acid
Sturgeon et al. (1980b)	Cd, Cr, Cu, Ca, Fe, Mn, Ni, Pb, Zn	APDC + Oxine/MIBK;Nitric Acid
Sugimae (1980)	Cd, Cu, Fe, Mn, Ni, Pb, V, Zn	NaDDC/Chloroform; Nitric Acid
Miyazake et al. (1982)	Cd, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn	APDC/Di-isobutyl ketone
McLeod et al. (1981)	Cd, Cu, Fe, Mo, Ni, V, Zn	APDC + DDC/Chloroform; Nitric Acid

b) Chelating Ion Exchange Methods

Reference	Metals	Chelating Ion Exchange Material
Sturgeon et al. (1981)	Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Silica immobilized 8-hydroxyquinoline
Sturgeon et al. (1980a), Bruland et al. (1979), Kingston et al. (1978)	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Chelex 100

c) Coprecipitation Methods

Reference	Metals	Precipitate
Boyle and Edmond (1975), Boyle and Edmond (1977), Klinkhammer (1980b), Klinkhammer (1982)	Cu, Ni, Cd, Fe	Cobalt APDC

method will probably not be widely adopted for routine use. The Chelex 100 methods are slower and are not able to extract colloidal or organically bound species of Cu and Ni from sea water.

A number of single element methods exist for Cr despite the fact that it can also be determined by multielement methods. The importance of the single element methods is that two of them can determine both species, Cr (III) and Cr (VI). Cranston and Murray's (1978) method relies on coprecipitation with $Fe(OH)_3$ while that of de Jong and Brinkmann (1978) utilizes solvent extraction with Aliquot-336. The single element methods for Cr do not seem to offer any significant advantages over the multielement methods of Sturgeon et al. (1980a). The preconcentration/GF-AAS methods of Rollemburg and Curtius (1982) and Burba et al. (1983) are indispensible for Ba and Be, respectively, since neither of these two elements can be satisfactorily determined by any other methods found in Volume 1. Single element methods exist for Mn and for V, but these do not seem to offer any significant advantages over the multielement methods which include Mn and V (Table 6.3).

6.4.3 Preconcentration Methods for Sediments

Preconcentration methods for sediments are uncommon and are usually not needed since most metals can be satisfactorily determined directly in the solutions resulting from digestion. Two preconcentration methods for sediments were found, however. Sturgeon et al.'s (1982) method uses solvent extraction to separate Cd, Pb, Cu, Ni and Co from the matrix. A pre-extraction is required to remove Fe and Al which would otherwise interfere, and this results in partial loss of Cd. The method of Sanzolone et al. (1979) also uses solvent extraction to separate Co, Ni and Cu. Interference from Fe and Al ions is avoided by masking them with fluoride.

These methods may be useful for bringing the low concentrations of Cd and Pb into the operating range for ICP or F-AAS and for reducing matrix interferences, but they add substantially to the analysis time. Cd and Pb can be satisfactorily determined by GF-AAS, and it is not necessary to remove matrix elements from dissolved sediments by solvent extraction to obtain satisfactory results.

6.4.4 Preconcentration Methods for Biota

6.4.4.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Preconcentrations are often applied to bring digested tissue samples into the analytical range of the instrument. As indicated in Section 6.3.4.1, a preconcentration step is needed for determining most metals in tissues by F-AAS or ICP. Preconcentration is also needed for determining certain metals in tissues by GF-AAS. Preconcentration separates the analytes from potentially interfering alkali and alkaline earth elements. Since the matrix of potentially interfering ions in tissues is very similar to that for sea water, the methods used in these two instances are also very similar. In general, preconcentration methods for sea water could be transferred with little difficulty to tissues and vice-versa. The preconcentration methods for tissues are summarized in Table 6.4. As with sea water, the main techniques are solvent extraction and chelating ion exchange.

RECOMMENDATIONS

The methods of Evans et al. (1978) and Agemian et al. (1980) which aspirate the organic solvent directly into the flame, are recommended for F-AAS. Okuno et al.'s (1978) method back-extracts the metals into nitric acid and is recommended for analysis by GF-AAS. None of these methods are appropriate for ICP, which would require complete removal of the organic solvent by evaporation and redissolution in nitric acid. Concentration factors of 10 are typical for these methods but factors of 100 could probably be attained judging from what is typical for seawater methods. It seems likely that solvent extraction methods developed for sea water could be readily applied to tissue digest solutions, thereby enabling higher preconcentration factors and more metals to be extracted.

The Chelex 100 method of Jones et al. (1982) is suitable for ICP or GF-AAS. When HF is used in the digestion, fluoride interferes with retention by forming soluble complexes with several elements. Jones et al.'s method has the advantage of covering a broad spectrum of metals and the disadvantage of being slower than most solvent extraction methods.

Table 6.4

Multielement Preconcentration Methods for Tissues

REFERENCE	METALS	CHELATING AGENTS/ EXTRACTION SOLVENTS
a) Solvent Extraction Methods		
Evans et al. (1978)	Cd, Pb, Ni	APDC + DDDC/4-methylpentan-2-one
Agemian et al. (1980)	Cd, Pb, Ni	APDC/MIBK
Okuno et al. (1978)	Cd, Pb, Cr	APDC/MIBK; Nitric Acid

REFERENCE	METALS	CHELATING ION EXCHANGE MATERIAL
b) Chelating Ion Exchange Methods		
Jones et al. (1982)	Al, Cd, Co, Cu, Mo	Chelex 100
Kingston et al. (1978)	Ni, Pb, V, Zn	

6.4.4.2 Methylmercury

There are two techniques for separating methylmercury from tissues for instrumental quantification by GC-EC, CV-AAS or GF-AAS. These are solvent extraction and steam distillation. Solvent extraction methods have been used for 20 years and are well established. There are two variations, that of Krenkel and Burrows (1975), which is standard, and the recent method of Hight and Capar (1983), which incorporates changes to make it simpler and faster. The Hight and Capar (1983) method was proven in a collaborative study and has been adopted by the Association of Official Analytical Chemists (A.O.A.C.). The steam distillation method of Collett et al. (1980) offers some advantages over the solvent extraction methods. It is faster and easier to perform; however, it may or may not result in as pure a product. The two methods are described in some detail in Section 2.4.4 of Volume I.

RECOMMENDATIONS

Comparing the complete methods, the solvent extraction/GC-EC methods of Krenkel and Burrows (1975) and of Hight and Capar (1983) seem to offer the most advantages. The steam distillation/CV-AAS method of Collett et al. (1980) seems to be equally sensitive but less selective. The steam distillation technique could perhaps be successfully combined with GC-EC, but this has not yet been proven.

6.5 Digestion Methods

6.5.1 General Considerations

The type of digestion method depends on the type of sample to be digested. The aluminosilicate minerals in sediments require HF to dissolve or else high temperature fusion with an alkaline flux. Organic matter can be mineralized with strong oxidizing acids such as HNO_3 and $HClO_4$. Alternatively, tissues can be ashed in a high or a low temperature furnace. The destruction of metal-organic complexes or organometallic compounds in interstitial waters can be accomplished under milder oxidizing conditions.

Within each broad category of sample type, there are variations in sample composition which necessitate variations in digestion procedure. For example, plant tissues sometimes contain silica, which requires HF to dissolve, while animal tissues usually do not. Sediments exhibit varying concentrations of organic matter and sometimes contain refractory minerals. Interstitial waters may or may not contain natural organic ligands which interfere with solvent extraction or chelating ion exchange preconcentration.

A decision that must be made in the analysis of sediments is whether to measure the total metal concentrations, the concentrations which are removed with strong concentrated acids or to measure the concentrations which are leached by some weaker and perhaps more selective form of chemical attack. The available choices and the considerations involved in making a choice are discussed in Section 6.5.3.1. Methods which measure particular metal species are sometime available; that option usually exists for As and Hg. Whether particular metal species are to be measured strongly influences the choice of digestion method.

The choice of a digestion method is influenced by the choice of instrumental technique, since the instruments are somewhat sensitive to the acids used, the viscosity of the solution, concentration of dissolved solids, etc. Whether a preconcentration step is needed to boost sensitivity and eliminate interferences also affects the choice of digestion method. The chemical properties of the metals to be determined may influence the choice of digestion method. The volatility of As and Hg compounds places restrictions on the conditions that can be employed in digestion procedures for these metals.

After sample type, type of attack (for sediments) or particular species, and instrumental technique have been considered in that order, final discrimination can be made on the basis of accuracy (including contamination, losses and completeness of digestion), cost (e.g., speed, ease, special equipment, etc.) and safety. It is sometimes difficult to evaluate methods according to these criteria because the information available is incomplete. Tables have been assembled to facilitate an objective choice when a wide variety of methods is available.

6.5.2 Interstitial Waters

6.5.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Bruland et al. (1979) found that Chelex 100 would not extract unreactive forms of dissolved trace metals from sea water and Danielsson et al. (1982) reported that their two-fold solvent extraction method manifested this interference in low salinity estuarine water samples. Although evidence of this problem occurring for interstitial waters is lacking so far, it seems likely to occur. **Therefore, it is recommended that either of the two successful digestive pretreatment procedures employed by Danielsson et al. (1982) be applied to interstitial water samples when total metal concentrations are to be determined.** These procedures consist of heating the sample in the presence of acid and H_2O_2. **When a digestive pretreatment is not used prior to solvent extraction, the determination should be called "extractable metal concentration".**

6.5.2.2 Arsenic

Since arsenic species are usually determined rather than the total concentration of arsenic, a digestion is not appropriate.

6.5.2.3 Mercury

Without oxidative pretreatment to liberate organo-mercury compounds or other unreactive forms of Hg, the CV-AAS methods of Olafsson (1983) and Bloom and Crecelius (1983) will measure the "easily reducible" fraction of Hg in the sample. For determining the total Hg concentration, Olafsson recommends oxidation with $KMnO_4 + K_2S_2O_8$ at 80C for 2 hrs. Bloom and Crecelius (1983) found that the bromine monochloride (cold) oxidation method of Szakacss et al. (1980) gave better results than the $KMnO_4 + K_2S_2O_8$ hot oxidation and the acid aging methods. The bromine monochloride method has a lower blank than the permanganate-persulphate method and is more effective than acid aging. A fourth alternative, the ultraviolet photooxidation method was successfully employed by Fitzgerald (1975).

6.5.3 Sediments

6.5.3.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Three kinds of digestions can be distinguished on the basis of whether it is desired to liberate all or part of the metals contained in the sediments:

a) **Total attack methods** determine the "total metal concentration", although incomplete dissolution is sometimes a problem for sediments containing refractory minerals or a large quantity of organic matter. Total attack procedures use HF in combination with other strong concentrated acids or else high temperature fusion with an alkaline flux and acid dissolution of the melt. Since determination of total concentration is intended, the accuracy of the determination can be assessed. This is important when comparison with a legal standard is necessary. The main disadvantage of most total attack methods is the unpopularity of HF.

b) **Strong attack methods** employ strong concentrated acids other than HF. Strong attack is usually seen as an alternative to total attack that is operationally less difficult but yields concentration values that are roughly comparable (for some metals in some sediments). One would normally expect almost complete dissolution of clay minerals, hydrous oxides and organic matter. The advantages of strong attack methods are: the use of HF is avoided; and they yield lower concentrations of salts, acids and interfering substances (such as Fe, Al and Si), resulting in a solution that is easier to quantify instrumentally. The disadvantage is that there is no way to assess accuracy. However, precision and reproducibility are usually good. Some workers feel that the results of strong attack are of limited value in assessing metal contamination as the attack removes some but not all of the metals incorporated in crystal lattices as well as metals derived from anthropogenic sources. Other workers feel that it is unnecessary to obtain complete digestion of all sedimentary components, including aluminosilicate minerals, since pollution effects usually occur on the surfaces of particles and in allochthonous particles.

c) **Moderate and weak attack methods** are usually aimed at determining the more readily available, biogeochemically important fraction of metals in sediments. They employ weaker and possibly more selective chemical attack agents, such as 0.05M EDTA, 2% sodium dithionite or 1M ammonium acetate. Three main purposes for using these kinds of digestion have evolved: to survey anthropogenic metal concentrations in sediments, to measure the 'biologically available' metal concentration, and for studying elemental partitioning. These methods can give some insight into the nature of the partitioning of elements among different sedimentary components (such as organic matter, carbonates and aluminosilicate minerals). They can be criticized on the grounds that they yield a value which is operationally defined and difficult to interpret. Perhaps a more important criticism is that they are less rugged than total or strong attack methods, i.e. more sensitive to variations in procedure.

RECOMMENDATIONS

a) Total Attack Methods

Acid attack methods are generally preferred to fusion attack for trace metals, because acid attack produces a solution which contains lower concentrations of acids and salts and which subsequently has lower blanks and fewer interferences. Instrumental technique is the most important factor affecting the choice of total acid attack method. **If F-AAS is to be used, it is sufficient to employ a conventional Teflon bomb or beaker digestion with HF plus aqua regia or HF plus nitric-perchloric acid followed by neutralization of the excess HF with boric acid. The methods of Loring and Rantala (1977) and Agemian and Chau (1975b) are of this type.** Dilution factors of 100-1000 are typical for this kind of digestion. If ICP or GF-AAS will be used however, it is preferable to substitute a careful evaporation step for treatment with boric acid to rid the solution of excess HF. Subsequent evaporations can be used to reduce the excess quantity of other acids used (such as perchloric) enabling final redissolution in 1M HCl. Thus the final solution is almost ideal for instrumental quantification. The methods of McLaren et al. (1981), Sturgeon et al. (1982) and Brzezinska et al. (1984) make use of careful evaporation steps in place of boric acid. A dilution factor as low as 50 can be achieved in this way. Whenever combinations of instrumental techniques are used to quantify different metals in the same solutions, the latter type of digestion method is recommended. Although the evaporation steps are more time consuming and difficult to perform, they are necessary to obtain the best results from GF-AAS and ICP.

Contamination of sediments during digestion is not likely unless for the very low level elements such as Pb, Cd, As and Hg. The use of Teflon bombs or Teflon beakers in a clean room or clean bench lessens the risk. Also the use of high purity acids and reagents in minimum quantities helps. Completion digestion can probably be effected with less acid in a Teflon bomb than in a Teflon beaker.

The older literature (e.g., Agemian and Chau, 1975c) alludes to possible loss of Cr as CrO_2Cl_2, but more recent experience with digestion methods which include evaporation steps seems to indicate that evaporation steps, carefully applied, do not cause any losses, even for arsenic. Thus, volatility does not seem to be a problem for total acid attack methods, and a Teflon bomb is not better than a Teflon beaker in this respect. Mercury, however, can be lost if the mixture of acids is not sufficiently oxidizing, and Si will be lost (as SiF_4) from an open vessel digestion with HF. Losses do not seem to be very limiting for the $LiBO_2$ fusion method of Owens and Gladney (1976); complete recovery of Ba, Be, Co, Cr, Cu, Mn, Mo, Pb, V and Zn was obtained from NBS fly ash.

Incomplete dissolution can sometimes be a problem when using total acid attack methods on sediments containing resistant minerals such as barite and chromite, or an especially high content of organic matter. The usual acid attack methods sometimes give values that are too low for Ba. **For determining barium in sediments, especially those containing barite $(BaSO)_4$), a fusion method (Owens and Gladney, 1976; Mahan and Leyden, 1983; Caravajal et al., 1983) is recommended.** Chromite may also resist complete dissolution by total acid attack methods (note the differing obtained results for Cr on BCSS-1 and MESS-1 by Sturgeon et al., 1982 and McLaren et al., 1981). Whenever incomplete dissolution of refractory minerals is a concern, a fusion method should be used, since fusion attack is more powerful. When sediments are very high in organic matter content, the $HNO_3 + HClO_4 + HF$ method of Hsu and Locke (1983) may be preferred, since the acid mixture used by this method is more oxidizing than that of Loring and Rantala or Agemian and Chau and is thus claimed to be more effective. From the standpoint of completeness of digestion, a Teflon bomb is probably superior to a Teflon beaker, - the bomb can be used at higher temperature and pressure. Grinding the sediments to a fine powder also increases the rate and extent of dissolution.

b) Strong Attack Methods

The alternative to total attack is usually strong attack with some combination of HCl, HNO_3, $HClO_4$ or H_2SO_4. The instrumental techniques are generally insensitive to which strong attack method is used. The choice of instrumental technique for strong attack follows the same logic as that for total attack; sensitivity is the main consideration. Strong attack methods are expected to liberate 10% - 100% of the total metal concentrations.

The aqua regia method of Agemian and Chau (1976) or the HNO_3 + HCl (9 + 1) method of Sinex et al. (1980) will suffice for most purposes. The more elaborate and time consuming procedure of Caravajal et al. (1983) seems unnecessary. For samples that contain unusually high amounts of organic matter, a more oxidizing combination of acids, such as $HClO_4$ + HNO_3 (1 + 1) (Agemian and Chau, 1976) or $HNO_3 + H_2O_2$ (10 + 3) (Krishnamurty, 1976) may be preferred. Sulphuric acid should perhaps be avoided, because sulphates may interfere with GF-AAS determination and precipitation of $PbSO_4$ is possible. **Grinding of sediments is not recommended when strong attack methods are used,** because grinding is unnecessary and introduces errors which tend to decrease precision and reproducibility.

c) Moderate and Weak Attack Methods

The choice of a specific method depends mainly on the objectives of the study, whether it is to survey anthropogenic metals, to measure the amount that is biologically available or to give information about strength of metal binding and association with particular sediment components. The results of moderate and weak attack methods are sensitive to sampling and sample storage procedures; the advice given by Thomson et al. (1980) should be followed. Because partial and selective extraction procedures sometimes are not very rugged, precise control over extraction time and temperature may be necessary. Long extraction times (8 hours) can also be used to minimize experimental error (Maher, 1984).

For pollution surveys, the 25% acetic acid leach of Loring (1981) and the 0.5M HCl leach of Chester and Voutsinou (1981) have proven to be effective. Agemian and Chau (1977) and Malo (1977) preferred 0.3-0.5M HCl for measuring authigenically formed metals in sediments. Luoma and Bryan (1981) recommended 1N HCl to provide a measure of nondetrital metal concentration. A selective extraction method which measures only anthropogenic and not natural forms of anthigenic metals is not available. Methods for measuring bioavailability is beyond the scope of the methods review; a single example is Luoma and Bryan (1981). Geochemical partitioning methods also were not given detailed coverage. Sources found in the Review are: van Valin and Morse (1982), Tessier et al. (1979), Engler et al. (1977), Luoma and Bryan (1981) and Maher (1984).

6.5.3.2 Digestion Methods for Arsenic in Sediments

a) **Total Attack Methods**

Three techniques are represented:

1) NaOH (or KOH) fusion (Goulden et al., 1981; de Oliveira et al., 1983)
2) $LiBO_2$ fusion (Mahan and Leyden (1983); Caravajal et al., 1983)
3) Acid digestion with HF (Agemian and Bedek, 1980; de Oliveira et al., 1983).

Accurate results were obtained for As in SRMs using all three techniques.

RECOMMENDATIONS

The acid digestion technique is preferred for arsenic for the same reasons that acid digestion is preferred to fusion attack for total metal determinations for other metals (convenience and lower salt/acid matrix). The use of an HF digestion for arsenic has the additional advantage that other elements can be determined on the same final solutions. The methods of Agemian and Bedek (1980) and de Oliveira et al. (1983) are compatible with both GF-AAS and HG-AAS (or HG-ICP) techniques. The use of a Teflon bomb is recommended to prevent loss of arsenic (III) halides (de Oliveira et al., 1983); otherwise oxidizing agents such as permanganate and persulphate are needed (Agemian and Bedek, 1980).

b) **Strong Attack Methods**

Numerous strong attack methods for determining arsenic in sediments are available.

1) $HCl + HNO_3$ (Caravajal et al., 1983)
2) $HNO_3 + HClO_4 + H_2SO_4$ (de Olievera et al., 1983; McLaren et al., 1981)
3) $HNO_3 + HClO_4$ (Vijan et al., 1976)

4) Aqua regia (Rubeska and Hlavinkova, 1979)

5) HCl (in sealed test-tubes) (Pahlavanpour et al., 1980).

Strong attack methods usually give a close approximation to the total arsenic concentration. Since accuracy cannot be assessed for strong attack methods, efficiency or convenience may facilitate a choice. **On that basis, the method of Pahlavanpour et al. (1980) excels, since it has the highest throughput and uses the least objectionable acid.**

c) Moderate and Weak Attack Methods

Maher's (1981) method is the only method in this category. It can be used to determine inorganic and methylated species of arsenic in sediments. The digestion procedure consists of an initial leach with 1M HCl followed by extraction with 0.1M NaOH + 11M NaCl. Most of the inorganic arsenic is extracted with the acid leach and methylated forms are found only in the alkaline solution.

6.5.3.3 Digestion Methods for Mercury in Sediments

The digestion is a critical step in the determination of total mercury in sediments. Strong attack methods are usually employed, and total attack is very uncommon. It is usually assumed that very little mercury is contained in aluminosilicate minerals, and it has been shown by Agemian and Chau (1975a) that HF is not necessary for determining total mercury in sediments. Essentially all of the mercury can be liberated using strong attack methods. However, some errors can still arise in strong attack digestion for mercury in sediments. For example, organomercury compounds may be volatilized by the high temperature required to oxidize the organic matter. Incomplete dissolution of cinnabar (HgS) is another source of error that has been suggested.

Elaborate strong attack digestion procedures have been developed for determining mercury in sediments to obviate these sources of error. The method of Agemian and Chau (1976) for example digests the sample with H_2SO_4 + HNO_3, and a trace of HCl is added to dissolve cinnabar. Then $KMnO_4$ and $K_2S_2O_8$ are added to ensure complete oxidation of the sample and of all forms of mercury. Hydroxylamine hydrochloride is then added to reduce the excess permanganate and persulphate, immediately before $SnCl_2$ is added to reduce mercuric ions to elemental mercury. Because this procedure uses so many reagents, it is more difficult to obtain a low blank.

More recently there has been a trend toward less complicated procedures which eliminate the auxiliary oxidization and the pre-reduction step. The method of Randlesome and Aston (1980), for example, merely uses HNO_3 + HCl. It is not known whether Randlesome and Aston's (1980) method can always be relied upon to give accurate results. The HNO_3 + HCl method of MacPherson et al. (1982) however, has been well validated using BCSS-1 and MESS-1. An auxiliary oxidant ($K_2Cr_2O_7$) is used, but the method is less complicated and less time consuming than Agemian and Chau's (1976) method.

RECOMMENDATIONS

The elaborate digestion procedure of Agemian and Chau (1976) can be used with the automated CV-AAS methods while MacPherson et al.'s (1982) simpler digestion procedure is appropriate for the manual CV-AAS methods.

6.5.4 Biota

6.5.4.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

There are basically, three techniques for digesting tissues:

1) wet oxidation,
2) high temperature ashing and
3) low temperature ashing.

Wet oxidation is by far the most commonly used technique. It is usually accomplished with a mixture of oxidizing acids such as $HNO_3 + HClO_4 + H_2SO_4$, carried out at relatively low temperatures, < 140°C, and is probably the safest with respect to volatilization of analytes. Since wet oxidation uses more acids than high and low temperature ashing, contamination from reagents is more problematic. Variations in the concentration of residual acid may be an interferent. High concentrations of residual acid may reduce the response of F-AAS and ICP. Incomplete dissolution is sometimes a problem, e.g. with high fat content samples. Safety can be a concern when employing wet oxidation methods.

High temperature ashing in a muffle furnace is second in popularity. Dissolution of the ash can be accomplished with less acid and therefore blanks are potentially lower. The high temperatures used (500-550°C) may volatilize some elements and fixation to solid components of the system can occur. Low temperature ashing is accomplished with atomic oxygen at low pressure in a special oxygen plasma furnace. Like high temperature ashing, low temperature ashing uses smaller quantities of reagents, and the lower temperature (100-140°C) is the main advantage of the technique. Its lower popularity can probably be attributed to the initial expense of the equipment, newness of the technique and its slowness relative to the muffle furnace technique.

Acid digestion of tissues is in some respects more difficult than sediments. Tissues are more variable in the problems they present, hence acid dissolution methods are more variable. Open or closed vessel digestions, single or successive additions of acids, with or without evaporation to dryness, constant temperature or temperature programs, various combinations of acids, etc. are some sources of variation in wet oxidation methods. Metal concentrations are generally lower in tissues than in sediments, by as much as one or two orders of magnitude, so contamination and reagent blanks are more problematic. Digesting tissues is more hazardous than digesting sediments. The evolution of CO_2 and H_2O makes digestion of tissues in closed containers difficult and there is more risk of an explosion.

In order to facilitate choosing a method from the variety that are available, the methods have been analyzed and classified according to certain criteria in Table 6.5. The criteria require some explanation. Sample type is an important factor because different kinds of samples to some extent require different kinds of digestions. Plant tissues sometimes require HF whereas animal tissues do not, and specialized methods have been developed for high fat content animal tissues. The method of digestion should be matched as closely as possible to the type of sample for which it is appropriate. The particular combination of acids used is given in the table. The original references must be consulted for the detailed procedures.

Which elements were measured, the instrument that was used and whether preconcentration was employed are given in the table. Digestion methods need not be considered as suitable only for the elements measured and the instrumental techniques used. The effectiveness of a digestion method can be judged on the basis of the extent to which its accuracy has been verified and, on the basis of the

TABLE 6.5

Digestion Methods for Plant and Animal Tissues

Reference	Jones et al. (1982)	Brzezinska et al. (1984)	Feldman (1974)	Agemian et al. (1980)	Borg et al. (1981)	Cary and Rutzke (1982)
Sample Type	Plant or animal	plant or animal	plant or animal	animal	animal	plant
Acids Used	$HClO_4 + HNO_3$ or $HClO_4 + HNO_3 + H_2SO_4$ (post-digestion of insoluble silicates with HF)	Plant: $HCl + HNO_3 + HClO_4 +$ HF (method a) or $HNO_3 + HClO_4 +$ HF (method b) Animal: $HNO_3 + HClO_4$ (methods a & b)	$HClO_4 + HNO_3 + H_2SO_4$.	$HNO_3 + H_2SO_4 + H_2O_2$	$HNO_3 + H_2O_2$	$HClO_4 + HNO_3 - H_2SO_4$ (post-digestion of insoluble silicates with HF)
Metals	alkali and alkaline earth, Fe, Mn, Al, P, Cd, Cu, Cr Co, Mo, Ni, V, Zn, As, Se, Sb	Ca, Mg, P, Fe, Mn, Al, Zn, Cu, Mo, Ni, Pb, Co, Cr, Cd, As, Se, Sb, Ag	Hg	Cu, Cr, Cd, Pb, Zn	Cu, Cd, Pb, Zn	Cr
Instrument/ Preconcentration	ICP/Chelex 100	ICP	CV-AAS	F-AAS/solvent extraction	GF-AAS	GF-AAS/$Fe(OH)_3$ coprecipitation
Effectiveness	- Accuracy verified for 7 SRMs - Results varied for binary and ternary acid mixtures and different metals - Most plant and some animal tissues required HF to obtain complete dissolution	- Accuracy verified for 5 SRMs - Complete dissolution assured with HF - Method a more sensitive but more open to contamination	- Accuracy verified for several SRM's - Immune to loss by volatization - low risk of contamination - Versatile procedure depends on sample type	- Accuracy verified for 3SRMs - Complete dissolution of high fat content fish tissue observed	- Accuracy verified by analysis of NBS bovine liver and by comparison with neutron activation analysis - Not suitable for volatile metals metals such as Hg and Se	- Accuracy vrified for 5 SRM's - Complete dissolution observed
Efficiency	- Kjeldahl flasks and heating rack - 2 step temperature program for ternary acid mixture	- Both methods use Teflon bombs in a pressure cooker - Method a: successive additions and evaporations; 3-4 hours - Method b: single addition, no evaporation; 2 hours	- Volumetric flasks fitted with air condensers on a hot plate; - Condenser assures retention of Hg vapour while allowing distillation of decomposition vapours - successive additions, temperature programming; takes 1-1/2 - 2-1/2 hours, can process 5-20 samples simultaneously	- Digestion tube in an aluminum heating block - Successive additions and evaporations - Includes a charring step	- Digestion tubes in an aluminum heating block - Successive additions and evaporations; 24 hours - Clean laboratory or filtered air hood recommended	- Digestion tubes - Time not given

information that is given on contamination, loss or completeness of digestion. Efficiency can be judged by how rapid, difficult or otherwise costly the method appears to be. This evaluation is also constrained by the amount of information contained in the methods reports. Because the effectiveness of a digestion procedure for a particular combination of metals determined, sample type and instrumental technique cannot be predicted, definitive recommendations are not given. It seems likely that most combinations will work; the obvious exceptions are few. Therefore, the recommendations are intended to be general, in order to provide a sufficiently broad scope to satisfy most needs.

RECOMMENDATIONS

For digesting animal tissues, a variety of different kinds of methods can be recommended. Methods which use combinations of $HNO_3 + HClO_4 + H_2SO_4$ are the most common. Feldman et al. (1974) and Jones et al. (1982) used these acids in Kjeldahl type flasks on a hot plate or heating rack. Five to 20 samples can be processed simultaneously in less than 3 hours. The air jacketed condenser used by Feldman (1974) is not necessary for most metals. Feldman's (1974) method has 3 variations and is therefore the most versatile. Brzezinska et al. (1984) used $HNO_3 + HClO_4$ in Teflon bombs heated in a pressure cooker. Depending on the concentration levels of the analytes, two variations, one which includes evaporation steps and one which does not, can be applied. If it is desired to avoid the use of $HClO_4$, methods employing $HNO_3 + H_2SO_4 + H_2O_2$ can be used. Agemian et al. (1980) and Borg et al. (1981) used combinations of these acids with digestion tubes in an aluminum heating block. The longer time required by Borg et al.'s (1981) method is not necessarily a disadvantage, since most of the time is unattended. Agemian et al.'s (1980) method includes a charring step which may be objected to by some.

For digesting plant tissues, the $HClO_4 + HNO_3 + H_2SO_4 + HF$ methods of Jones et al. (1982) and Cary and Rutzke (1983) can be recommended. The $HNO_3 + H_2SO_4 + H_2O_2$ method of Arafat and Glooschenko (1981) has the drawback that complete dissolution was not obtained, probably because HF was not used.

6.5.4.2 Digestion Methods for Arsenic in Tissues

a) For Determining Total Arsenic Concentrations

There are many methods (11) available for determining total arsenic concentrations in biological tissues. Most of these gave accurate results for arsenic in SRMs. One was not properly validated and another gave results that were too low.

It is controversial whether wet or dry ashing techniques should be used for determining As in samples of marine origin. Both techniques have been applied successfully. Some evidence has been advanced that if a wet digestion method is used which does not have sufficient oxidizing power to keep arsenic in the pentavalent oxidation state, loss of As might result.

RECOMMENDATIONS

For animal tissues, the nitric-perchloric-sulphuric acid methods of Thiex (1980) and Agemian and Thomson (1980) are recommended. This is probably the most popular combination of acids for digesting animal tissues, and it is sufficiently oxidizing to prevent volatilization of As (III) compounds. The effectiveness of the 2 methods has been well validated. The method of Agemian and Thomson (1980) which utilizes the aluminum heating block technique, is probably the more efficient. The methods are suitable for use with HG-AAS (Agemian and Thomson, 1980) and solvent extraction/GF-AAS (Thiex, 1980). The dry ashing method of Evans et al. (1979) is a viable alternative to the two wet ashing methods. $Mg(NO_3)_2$ is used as an ashing aid and to ensure complete recovery of As. It has been well validated and is suitable for HG-AAS.

The recommended methods for plant tissues are the $H_2SO_4 + HNO_3 + H_2O_2$ methods of Kuldvere (1980) and Arafat and Glooschenko (1981). The latter obtained accurate results for As in three SRMs and is suitable for HG-AAS. The procedure, which uses Erlenmeyer flasks and a hot plate, would be more efficient if modified to use digestion tubes in an aluminum heating block. Kuldvere's (1980) method, which was applied to seaweed, uses the aluminum heating block technique and is also suitable for HG-AAS.

b) For Determining Arsenic Species

There are three methods available for determining arsenic species (As III, As V, monomethylarsonic acid and dimethylarsinic acid) in biological tissues. These employ selective extractions rather than acid digestion to separate arsenic compounds from the biological matrix. Andreae (1978) extracted homogenized tissues with 2M HCl. Maher (1981) found that extraction with 0.1M NaOH yielded better recoveries than 1M NaOH, 7.5M HCl or water and did not degrade the methylated species to inorganic arsenic. The methylated species accounted for a significant fraction of total As in seaweed. Aggett and Kadwani (1983) extracted homogenized plant tissues with distilled water, methanol and ethanol. Inorganic arsenic was extracted with water, while significant amounts of methylated species were extracted with methanol or ethanol.

RECOMMENDATIONS

It is clear from these studies that the above mentioned extractants can be used to separate arsenic species from homogenized tissues and that the extractions are somewhat selective for the different species. As in the use of selective or partial extraction methods for sediments, the task of recommending partial extraction methods that are appropriate for particular purposes falls outside the scope of this guide.

6.5.4.3 Digestion Methods for Mercury in Tissues

The choice of digestion method for determining total mercury in tissues appears to be crucial for obtaining accurate results. A variety of different techniques are available, and they fall into two categories - those which effect complete oxidation of organic matter and those which do not. Another distinction which can be made is whether oxidation is accomplished under acidic or basic conditions. All of the methods except one were applied to fish tissues.

The available methods are listed below:

a) Complete Oxidation Methods

Feldman (1974)	$HClO_4 + HNO_3 + H_2SO_4$
Agemian and Chau (1975b, 1976)	$H_2SO_4 + HNO_3$; $KMnO_4 + K_2S_2O_8$
Agemian and Chau (1978)	$H_2SO_4 + H_2O_2$; $KMnO_4 + K_2S_2O_8$
Velghe et al. (1978a)	$H_2SO_4 + KMnO_4$
Chapman and Dale (1982)	1.5M KOH; $KMnO_4$; H_2SO_4

b) Incomplete Oxidation Methods

Tong and Leow (1980)	30% NaOH + 0.6% NaCl
Velghe et al. (1978b)	H_2SO_4

The complete oxidation methods employ a pre-reduction step (using hydroxylamine or oxalic acid) and are therefore generally more time consuming. The elaborate procedure of Feldman (1974) appears to be safest with respect to loss of mercury by volatization during digestion. Only the methods of Feldman (1974), Chapman and Dale (1974) and Tong and Leow (1980) have been properly validated using SRM's. On this basis it appears that a rigorous oxidative digestion is not necessary to obtain accurate results. The method of Tong and Leow (1980) appears to be as effective, and it is significantly faster.

RECOMMENDATIONS

The method of Feldman (1974) is preferred, since it seems to be the most effective (in terms of contamination, loss, completeness of digestion) yet is probably not more time consuming than the other complete oxidation method that has been successfully validated (Chapman and Dale, 1982). The method of Tong and Leow (1980) is less time consuming, but it may objected to by some because it is not rigorous. Nevertheless it has been well validated.

6.6 Cost of Analysis

Approximate costs for the main types of analyses are given in Table 6.6. These are based on 1984 prices obtained from several commercial laboratories in the

Vancouver-Victoria, B.C. area. For AAS, the cost per sample depends on the number of elements determined. For ICP however, a flat rate is charged for 20 or 30 elements determined. Thus it is much cheaper to do a large number (20-30) of elements by ICP (as reflected by this table), the cross-over being in the neighborhood of 3-5 elements. The ranges reflect the variability among laboratories, which is large. The methods used by the different laboratories varies somewhat which accounts for some of the variability.

Table 6.6
Per Sample Cost of Analysis

	Group I Metals			As	Hg
	F-AAS	GF-AAS	ICP-AES	HG-AAS	CV-AAS
Sediments	\$60-100[1]	\$90-115[1]	\$15-50[1,4]	\$10-25	\$10-25
Biota	\$60-100[2]	\$90-115[2]	\$15-50[2,4]	\$10-25	\$10-25
Interstitial Water	-	\$50- 75[3]	-	-	-

1 Total acid digestion and direct determination of the 10 Group I metals.

2 Acid digestion and direct determination of the 10 Group I metals. For a method which includes a preconcentration step, add \$10-20.

3 Solvent extraction preconcentration and GF-AAS determination of 5 Group I metals.

4 ICP determination includes 10 Group I metals plus 10-20 other elements.

7.0 METHODS FOR THE ANALYSIS OF ORGANICS

This section outlines selection criteria for choosing appropriate methods for the determination of selected organic compounds in marine sediments and benthic organisms. The following types of analyses are included:

1. Total Organic Carbon (TOC)
2. Chemical Oxygen Demand (COD)
3. Petroleum Hydrocarbons
4. Oil and Grease
5. Phenols
6. Chlorophenols
7. Chlorinated Hydrocarbons (Pesticides and PCBs)

The recommendations given are based on the review of methods in Volume 1.

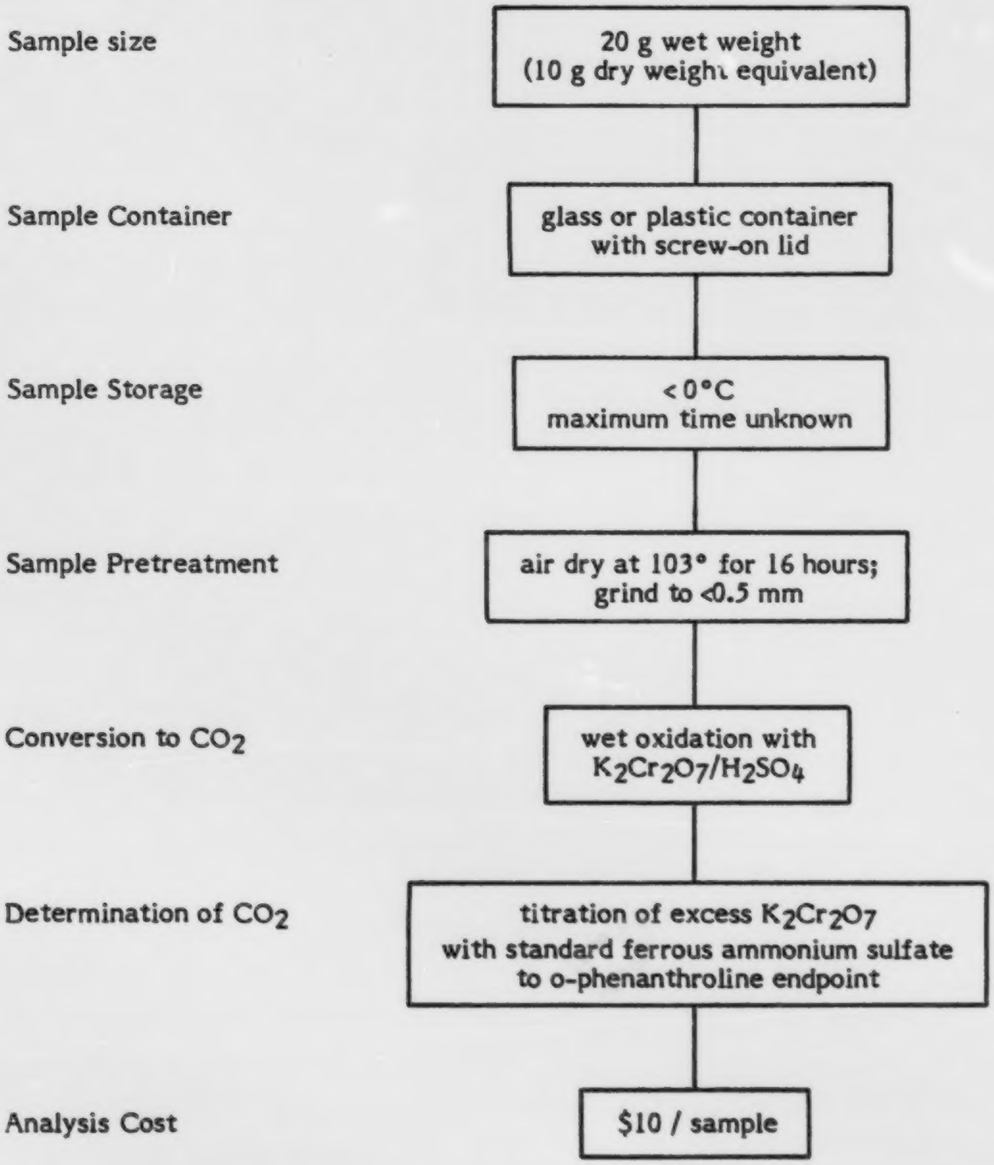
7.1 Total Organic Carbon (TOC)

TOC analyses are two step procedures involving the conversion of organic carbon to CO_2 and measurement of either the quantity of oxidant consumed for the conversion or the CO_2 evolved. Organic carbon can be converted to CO_2 by either wet oxidation (with potassium dichromate/sulfuric acid or potassium persulfate) or dry combustion (heating at 550 - 1600°C in the presence of O_2). The evolved CO_2 can be determined gravimetrically, volumetrically, by infrared absorbance or by thermal conductivity measurements. Analytical methods for TOC determinations are reviewed in Section III-3.2, Volume 1.

Two methods are recommended for TOC analysis of sediments: a low cost method (wet oxidation-titration) and a high accuracy method (acid digestion - dry combustion). The detection limit of the first method is 1 $mg \cdot g^{-1}$ while that of the second is approximately 250 $ug.g^{-1}$. These methods are outlined in Table 7.1.1 and 7.1.2 respectively and discussed below.

Table 7.1.1

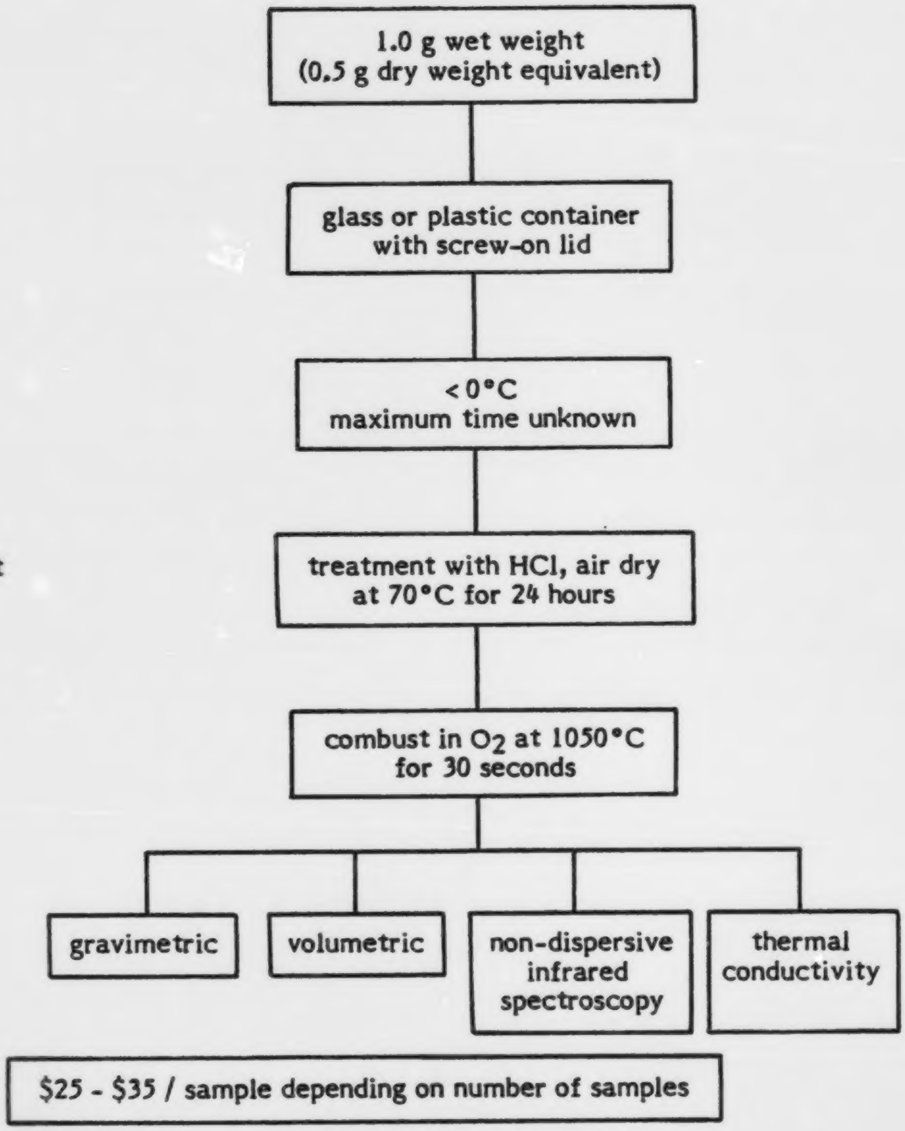
Determination of TOC in Sediments by Wet Oxidation/Titration



Sample size	20 g wet weight (10 g dry weight equivalent)
Sample Container	glass or plastic container with screw-on lid
Sample Storage	< 0°C maximum time unknown
Sample Pretreatment	air dry at 103° for 16 hours; grind to <0.5 mm
Conversion to CO_2	wet oxidation with $K_2Cr_2O_7/H_2SO_4$
Determination of CO_2	titration of excess $K_2Cr_2O_7$ with standard ferrous ammonium sulfate to o-phenanthroline endpoint
Analysis Cost	\$10 / sample

Table 7.1.2

Determination of TOC in Sediments by Acid Digestion/Dry Combustion



Sample Size	1.0 g wet weight (0.5 g dry weight equivalent)
Sample Container	glass or plastic container with screw-on lid
Sample Storage	< 0°C maximum time unknown
Sample Pretreatment	treatment with HCl, air dry at 70°C for 24 hours
Conversion to CO_2	combust in O_2 at 1050°C for 30 seconds
Determination of CO_2	gravimetric volumetric non-dispersive infrared spectroscopy thermal conductivity
Analysis Cost	$25 - $35 / sample depending on number of samples

7.1.1 Sample Requirements

Up to 20 grams wet weight of sediment is required for TOC determination by the wet oxidation-titration procedure (EPS, 1979). The exact sample size required is determined by its organic carbon content (10 to 25 mg of organic carbon per sample aliquot analyzed). Approximately one gram wet weight of sediment is required for TOC analysis by the acid digestion-dry combustion method.

Glass or plastic containers can be used for sample storage (EPS, 1979; Plumb, 1981). Samples should be stored moist at 4°C to minimize loss of organic compounds through volatilization. Maximum times for sample storage are unknown but should be minimized to avoid loss due to chemical or biological oxidation (Plumb, 1981). One month is suggested as a reasonable storage time.

7.1.2 Wet Oxidation - Titration Procedure

The Walkley-Black wet oxidation-titration method is recommended for use as an inexpensive determination of the TOC content of sediments (EPS, 1979). The advantages of this procedure include its low analysis cost, low capital cost (only standard laboratory equipment is required) and widespread historical use. However, results by this method may be an inaccurate indicator of absolute organic carbon content of the sediment and should be considered only as a procedurally-defined quantity. EPS recommends designation of results as "Organic Carbon, chromic acid oxidation values". Inaccuracies arise from

i) the incomplete oxidation of organic carbon (between 70 and 90% complete, depending on the refractory carbon content of the sediment);

ii) standardization difficulties (the organic carbon may be at a different overall oxidation state than the organic carbon used to standardize the titration); and

iii) the non-specificity of the oxidation towards carbon (oxidizable interferences include iron (II), manganese (II), sulfide, and to some extent, chloride ions).

The standardization and non-specificity errors can be minimized by directly measuring the CO_2 evolved by the wet oxidation. This however requires special equipment or adaptation of commercial instruments adding significantly to the capital cost, the analysis cost and the inconvenience. Such a modification is not warranted for this analysis as it eliminates the advantages of the wet oxidation technique while ignoring the errors due to incomplete oxidation of the organic carbon in the sample.

The EPS procedure calls for drying of the sediment sample before analysis. The drying technique is not critical (freeze-drying, air-drying or oven-drying at temperatures between 60 and 180°C are acceptable). The drying step may result in some loss of volatile organic compounds from the sample and caution should be exercised if such components are expected to be major constituents of the organic material in the sediment.[1]

The organic carbon in the sample is oxidized by treatment with a potassium dichromate/sulfuric acid solution. This reagent produces higher oxidation efficiencies than potassium persulfate. The excess oxidant is measured by titration with standard ferrous ammonium sulfate to an o-phenanthroline endpoint. The organic carbon content of the sample is calculated from the number of equivalents of potassium dichromate used.

7.1.3 Acid Digestion - Dry Combustion Procedure

A dry combustion procedure is recommended for accurate determination of the organic carbon content of sediment samples. Such a method is more specific for carbon as it measures the quantity of evolved CO_2 directly and complete oxidation of organic carbon can be achieved by selection of appropriate combustion conditions.

To maximize the accuracy of the TOC value, organic carbon should be determined directly rather than by subtraction of inorganic carbon values from total carbon values. Two methods of differentiating organic carbon from inorganic carbon are used:

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[1] Total organic carbon is defined as carbon in non-volatile organic compounds (Plumb, 1981).

i) prior removal of inorganic carbon (i.e., carbonate and bicarbonate) from the sample by acidification or

ii) a differential combustion method in which organic compounds are selectively oxidized under mild conditions.

The combustion of inorganic and organic carbon at overlapping temperatures may cause significant error in a differential combustion method. Therefore removal of inorganic carbon by acidification with a non-oxidizing acid such as H_3PO_4 or HCl (Plumb, 1981) is the recommended method. The acidification method provides a good partition between organic and inorganic carbon but has some potential for experimental error (sample can be lost by frothing or acid-soluble organic carbon (SOC) may be lost by leaching through permeable crucibles or if a filtration is carried out). The EPA method (Plumb, 1981) details the steps required to avoid such errors (slow addition of acid, drying rather than filtration).

Dry combustion is carried out in a stream of O_2 using an induction or a resistance furnace (Plumb, 1981). Combustion temperatures ranging from 550 - 1600°C have been reported in the literature. Gibbs (1977) recommends combustion at 1050°C or greater for at least thirty seconds.

Dry-oxidation TOC analyses are generally carried out using commercially available instrumentation that couples a combustion system to a CO_2 measuring system. Such systems can be volumetric, gravimetric, thermal conductivity or infrared absorbance. All provide accurate measurement of evolved CO_2 and selection should be made on the basis of availability and cost of the various instruments. Volumetric, gravimetric and thermal conductivity methods require prior separation of interfering gases (such as H_2O, SO_2 and Cl_2) while infrared measurements are specific to CO_2.

7.2 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is a procedurally-defined quantity used as an indicator of organic material in sediments. The sediment sample is digested with a strong oxidizing agent at elevated temperatures and low pH and the amount of oxidant consumed is measured and related to an equivalent amount of O_2. **A standard method for COD determinations on sediments has been published by the EPA (Plumb, 1981) and is recommended for use.**

7.2.1 Sample Handling and Storage

Approximately 2 grams wet weight of sediment is required for COD analysis. Samples are stored wet in precleaned (detergent wash, distilled water rinse) glass or plastic containers, minimizing air contact and storage headspace to prevent air oxidation. Recommended storage temperature is 4°C with a time limit of one week (Plumb, 1981). No pretreatment or drying of the sediment is required.

7.2.2 Method of Analysis

As COD is a procedurally-defined quantity, the standard method (EPA: Plumb, 1981) should be followed exactly. The sediment is refluxed with a known amount of potassium dichromate ($K_2Cr_2O_7$) solution. The excess $K_2Cr_2O_7$ is measured by titration with standard ferrous ammonium sulfate to a ferroin endpoint. The $K_2Cr_2O_7$ consumed is converted to an equivalent quantity of O_2 and called COD.

7.3 Petroleum Hydrocarbons

Many types of analyses for petroleum hydrocarbons can be performed on environmental samples, ranging from a qualitative analysis in which the samples are simply screened for the presence of petroleum compounds to accurate quantification or detailed compositional description of the petroleum present. As shown in Table 7.3.1, several techniques can be used to obtain each of these various types of information on sediment and biota extract solutions. The choice of analytical method for a particular analysis is therefore based on several factors which are predetermined by the user of the analytical results and are dependent on the goals of the analysis program being carried out. These factors include:

1) accuracy
2) sensitivity (or detection limit)
3) the compositional information required and
4) the cost.

The analytical techniques used in petroleum analysis are rated in terms of these factors in Table 7.3.1 and discussed in detail in Section III-3.4 of Volume I.

Table 7.3.1

Methods for Petroleum Hydrocarbon Determination Rated According to Sensitivity, Accuracy, Level of Compositional Information and Cost

METHOD	QUANTITATION			COMPOSITIONAL INFORMATION	COST*
		Accuracy			
	Relative Sensitivity	Without Extract Cleanup	With Extract Cleanup		
gravimetry	low	low	high	none	$ 10
infrared spectroscopy	low	low	low	aliphatic content, functional group analysis	$ 15
microgravimetry	moderate	low	high	none	$ 15
ultraviolet spectroscopy	moderate	low	low	aromatic content	$ 15
UV-fluorescence spectroscopy	high	moderate	moderate	aromatic content	$ 15
gas chromatography/ flame ionization detector	high	low	high	boiling point distribution OEP, pristane/phytane ratio, n-alkane/isoprenoid ratio, major and minor component identification, alkylated PAH distribution	$40-100
gas chromatography/ mass spectrometric detection	high	moderate	high		$80-200

* Based upon analysis for petroleum hydrocarbons of one fraction of an extract dissolved in a solvent compatible with the method, but does not include the cost of sample extraction, clean-up and fractionation. Instrumental calibration costs are averaged over a suite of 5 samples. Where composition information is also generated, the cost includes data collection for a range of options, from minimal compositional information (just total petroleum hydrocarbons) to a full suite of parameters typically of interest.

A series of specific sensitivity/accuracy goals for the quantification of petroleum hydrocarbons in sediment and biota is presented in Table 7.3.2, along with the instrumental techniques which can be used to achieve each of these goals. Similarily, the techniques which can be used to provide compositional information on the petroleum in extracts are shown in Table 7.3.3 The recommended procedures for each sensitivity/accuracy and compositional analysis goal are discussed in the following sections.

7.3.1 Sample Requirements

Approximately 50 g wet weight of sediment (25 g dry weight equivalent) or 50 g wet weight of tissue (5 - 15 g dry weight equivalent) is required for petroleum hydrocarbon analysis. Although each individual method specifies a particular sample weight requirement (ranging from 1 g to 200 g), this is mostly a matter of convenience. It has been suggested (Hoffman et al., 1979) that subsampling errors are a primary contributor to poor correlations between petroleum hydrocarbon determinations by various methods. Due to the inhomogeneity of environmental samples, it is recommended that reasonably large samples (of approximately the same size) be taken for analysis and that a subsampling of the (liquid) extract be carried out if required for subsequent steps of the analysis. This will decrease sample size contributions to any variability in analytical results between the methods.

7.3.2 Storage Containers and Storage Conditions

Several types of containers are suitable for storing sediment samples for hydrocarbon analysis. Glass, aluminum, Teflon and stainless steel containers can be used but plastic containers and utensils and rubberized or waxed lids must be avoided (Clark and Brown, 1977). **Glass jars with teflon-lined lids are recommended.** However, aluminum foil-lined lids may be sufficient for analyses that do not require a high degree of accuracy. Although aluminum foil is rapidly deteriorated by the sediment (exposing the plastic lid and glue to the sample) it is less expensive and readily available. Glass containers and all glassware used in the analysis must be precleaned with detergent, rinsed with acetone and heated at 350°C for sixteen hours (Clark and Brown, 1977). Teflon or aluminum foil used for lining lids must also be washed in a non-polar solvent such as hexane.

Table 7.3.2

Methods for the Determination of Total Petroleum Hydrocarbon Content in Sediment and Biota Extracts

Goal	Techniques	Potential Application
A. Screening	gravimetry, IR, UV, UV-fluorescence	- identification of contaminated samples, i.e. determination of a real extent of damage
B. Low Sensitivity/ Low Accuracy	IR, UV, UV-fluorescence, microgravimetry	- estimation of level of contamination
C. Low Sensitivity/ Moderate Accuracy	microgravimetry, UV, UV-fluorescence, GC/FID, GC/MS	- monitoring trends in contamination levels as a function of time and/or location
D. High Sensitivity/ Moderate Accuracy	GC/FID, GC/MS	- monitoring trends in low level contamination as a function of time and/or location
E. High Sensitivity/ High Accuracy	GC/FID, GC/MS	- baseline levels
F. Low Molecular Weight Hydrocarbons	GC	- measuring levels of contamination

Table 7.3.3

Methods for Obtaining Petroleum Hydrocarbon Compositional Information on Sediment and Biota Extracts

Goal	Techniques	Potential Application
Aromatic content	UV, UV-fluorescence, GC/MS GC/FID on aromatic fraction, gravimetry on aromatic fraction	- determination of source of input e.g. petroleum pollution vs. incomplete combustion of fossil fuels
Aliphatic content	IR, GC/MS, GC/FID on aliphatic fraction, gravimetry on aliphatic fraction	
Boiling point distribution or distillation curve	GC/FID	- identification of source as refined product - determination of degree of weathering of contaminant i.e. new vs. chronic pollution
OEP	GC/FID, GC/MS	- distinction of biogenic from anthropogenic inputs
Pristane/phytane ratio	high resolution GC/FID, GC/MS	- distinction of biogenic from anthropogenic inputs
n-alkane/isoprenoid ratio	high resolution GC/FID, GC/MS	- determination of degree of weathering of contaminant i.e. new vs. chronic pollution
major component analysis	GC/FID GC/MS	- determination of source of input - determination of degree of weathering of contaminant - distinction of biogenic from anthropogenic inputs
minor component analysis	GC/MS	- determination of source of input - determination of degree of weathering of component
parent and alkylated aromatic homolog distribution	GC/MS	- determination of source of input e.g. petroleum pollution vs incomplete combustion of fossil fuels

Biota samples should be wrapped tightly with hexane-rinsed aluminum foil and stored in a container (not plastic) with a tight fitting lid (Clark and Brown, 1977). Some people have recommended rinsing of the biota with a solvent to remove surface petroleum, but this should not be necessary for benthic biota and may even result in some loss of petroleum hydrocarbons.

Both sediment and biota samples should be maintained tightly capped and in a frozen state until analysis (Clark and Brown, 1977). The effect of storage time on sample integrity is unknown but it has been suggested as a contributing factor to variability of results in interlaboratory comparisons. **It is therefore recommended that sample storage time before analysis be minimized and be less than six months for maximum accuracy and precision of results.**

No pretreatment of the sample is required. Samples are thawed immediately prior to analysis and analyzed wet or dry. There is some concern over loss of petroleum compounds during air-drying, oven-drying or freeze-drying procedures so drying is avoided when maximum accuracy is required. Drying of the samples is discussed below for each analysis procedure recommended.

7.3.3 Semi-Quantitative Screening for Petroleum Hydrocarbons

A rapid and inexpensive semi-quantitative analysis can be performed on sediment and biota samples to detect the presence of petroleum hydrocarbons. The recommended analysis procedure for sediment samples is outlined in Table 7.3.4 and the procedure for biota in Table 7.3.5 . These methods are suitable for detection of moderate and high levels of petroleum contamination (greater than approximately 50 $\mu g \cdot g^{-1}$ in sediment and tissue). Other methods must be used if lower detection limits are required (see Sections 7.3.2 to 7.3.4).

7.3.3.1 Detection of Petroleum Hydrocarbons

Gravimetry, infrared, ultraviolet (UV) spectroscopy or UV-fluorescence can be used to detect petroleum compounds in sediment and biota extracts. Although these techniques are comparable in cost and availability, gravimetry and infrared spectroscopy are unable to distinguish petroleum from biogenic and diagenetic material in the extract. This distinction can be made if the extract solution is subjected to cleanup procedures prior to the petroleum determination. The infrared method is also susceptible to interference from water, imposes limitations on the

Table 7.3.4

Screening Sediments for Petroleum Hydrocarbons

Sample Size	50 g wet weight (25 g dry weight equivalent)
Sample Container	precleaned glass bottle (detergent wash, acetone rinse, heat at 350°C for 16 hours) with screw-on lid with foil lining
Sample Storage	< -10°C < 12 months
Sample Pretreatment	air dry
Extraction	sonication in non-fluorescing solvent e.g., hexane, cyclohexane
Extract Cleanup	none
Detection	UV-Fluorescence
Analysis Cost	$30-50/sample

Table 7.3.5

Screening Biota for Petroleum Hydrocarbons

Sample Size	50 g wet weight of tissue (5-15 g dry weight equivalent)
Sample Container	wrap in hexane-rinsed aluminum foil, store in closed container
Sample Storage	< -10°C < 12 months
Sample Pretreatment	none
Extraction	blend tissue with anhydrous Na_2SO_4 and pentane
Extract Cleanup & Fractionation	none
Detection	UV-Fluorescence
Analysis Cost	$30-50/sample

choice of extraction solvent and is strongly biased in favour of saturated hydrocarbon compounds. UV spectroscopy and UV-fluorescence are more specific for petroleum as they are biased in favour of aromatic compounds. Both UV and UV-fluorescence measurements can be obtained on the whole (uncleaned, unfractionated) extract, thus reducing the overall analysis cost. However, fluorescence is the preferable technique as it is more specific and can detect lower levels of petroleum contamination.

The application of UV-fluorescence to the determination of petroleum hydrocarbons in sediment and biota extracts is described by Hoffman *et al.* (1979) and Fong (1976) respectively. Synchronous scanning UV-fluorescence measurements should be made if the instrumentation is available as it has the ability to differentiate background pigment contributions to the observed fluorescence. Otherwise fixed wavelength UV-fluorescence measurements can be obtained. These instruments are less expensive and more commonly available than the synchronous scanning instruments, but there is more possibility of interference from co-extracted material when fixed wavelength measurements are used.

7.3.3.2 Sediment Extraction

Pre-drying of the sediment is recommended for the semi-quantitative screening analysis. Loss of hydrocarbons during the drying of the sediment is likely to be insignificant to the outcome of the analysis if it is carefully carried out. Air-drying is the recommended procedure (Overton and Laseter, 1980). The analysis of pre-dried sediment permits the use of a non-polar solvent for extraction, minimizing the amount of co-extracted polar lipid material and facilitating adjustment of extract solution volumes prior to the detection step.

Any extraction technique listed in Table III-3.12 Volume 1 can be used for a screening analysis. Some of these methods, while very efficient at extracting petroleum compounds from the sample matrix, are time consuming and contribute significantly to the total analysis time and cost. They may also result in large quantities of co-extracted material which interfere in the subsequent detection of petroleum hydrocarbons. Selection of an extraction method is therefore based on speed and cost of analysis rather than on efficiency of extraction. The method of Tan (1979) satisfies these requirements. Pre-dried sediment is extracted by sonication in a non-polar solvent such as cyclohexane. The choice of extraction solvent is dependent on the instrumental technique used for detection of petroleum hydrocarbons.

Solvents for UV fluorescence analysis are limited to those which do not fluoresce or quench fluorescence and saturated hydrocarbons (usually hexane, isooctane or cyclohexane) are recommended. If other solvents are used for extraction, such as freon or dichloromethane, they must be exchanged to a suitable hydrocarbon solvent prior to UV-fluorescence analysis.

When UV-fluorescence is used for detection of petroleum hydrocarbons, a prior extract cleanup step is not required. However, extract cleanup will increase the specificity of the analysis and may be included in the analysis procedure for a small additional cost (see Section 7.3.4.3). Without cleanup other fluorescing biogenic materials (pigments, phospholipids, etc.) may be detected and reported as petroleum contamination.

7.3.3.3 Biota Extraction

Homogenized tissue samples can be extracted wet using a combined polar:non-polar solvent or they can be dried and then extracted by any of the methods listed in Table III-3.13 of Volume I. Such methods result in substantial quantities of co-extracted biological material which interfere with most hydrocarbon determination techniques so that cleanup is required before analysis. It is more convenient to selectively extract the hydrocarbons from wet tissue with a non polar solvent. Drying and extraction are accomplished in one step by blending the thawed biota with pentane and anhydrous sodium sulfate (Gay et al., 1980). Although this is not the most exhaustive extraction procedure, it is a rapid and inexpensive method of extracting petroleum hydrocarbons from tissue samples.

As with sediments, extract cleanup is not required prior to UV-fluorescence detection of the petroleum hydrocarbons. Extract cleanup will increase the specificity of the analysis and may be included in the procedure if required (see Section 7.3.4.3). Potential interferences in the fluorescence detection are even greater with biota samples than with sediments.

7.3.4 Quantitative Analysis Procedures Providing Low Accuracy Determination of Petroleum Hydrocarbons

A rapid and inexpensive analysis can be performed on sediment and biota samples to estimate their level of petroleum hydrocarbon contamination. Procedures employing either UV-fluorescence or microgravimetric determination of the petroleum hydrocarbons can be used for this purpose and are outlined in Tables 7.3.6 (for sediment) and 7.3.7 (for biota). The UV-fluorescence and the microgravimetric procedures are similar in cost and performance and method selection should be based on availability of equipment (i.e. fluorescence instrument or microgravimetric balance).

7.3.4.1 UV-Fluorescence Determination

This method is similar to that recommended above for screening samples for petroleum hydrocarbon content (Section 7.3.1). A rapid extraction is carried out and UV-fluorescence measurements are obtained directly on the extract without any prior cleaning or fractionation (Hoffman et al., 1979; Fong, 1976). However, in this case, the fluorescence response must be converted to petroleum concentrations.

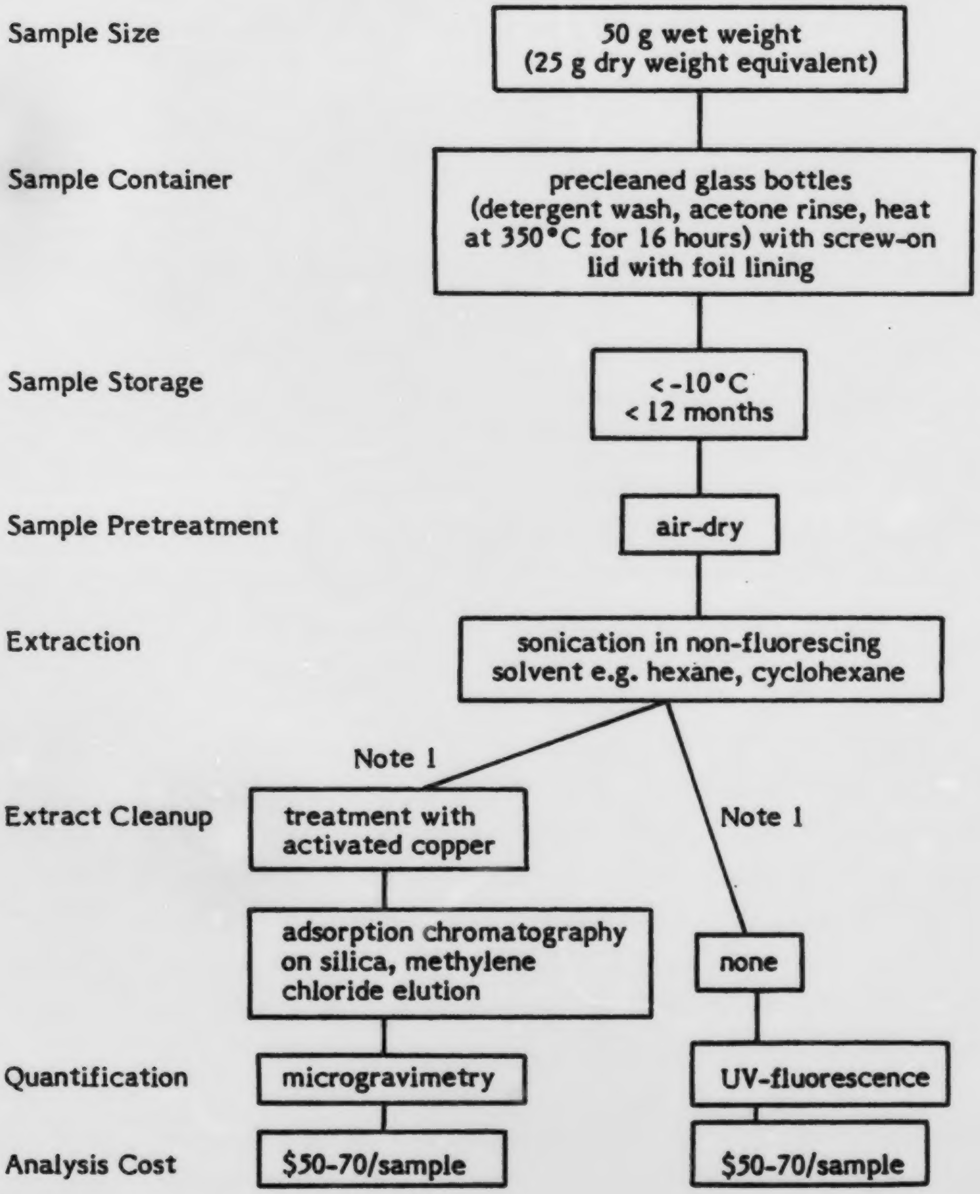
The key to quantification of petroleum compounds by UV-fluorescence spectroscopy is the choice of calibration standard. Ideally the standard should be identical in molecular composition to the petroleum in the samples, but this is very difficult with sediment and biota in which the petroleum may be fractionated or degraded or from a variety of sources. An arbitrary reference oil may be used to calibrate the UV-fluorescence response and the results reported as "equivalent oil concentrations". This procedure is most useful in cases in which a target oil is available, i.e., in oil spill situations, but is not commonly used otherwise.

An alternative method is the use of a chrysene solution for calibration and the results reported as "chrysene equivalents" (Cretney and Wong, 1974). Results using these calibration methods may not necessarily agree with each other or with results by more accurate quantification techniques. This may be especially true for sediment and biota extracts in which the petroleum could be variably fractionated. Quantitative analysis using a UV-fluorescence technique is therefore most useful when results are to be used only for comparison with other results by the same method and when absolute petroleum concentrations are not required.

The use of a rigorously quantified sediment or biota extract as the calibration standard is potentially more accurate than either of these calibration techniques and may be useful when estimations of absolute concentrations are desired or when variable fractionation of the petroleum is suspected. The analysis procedures required to rigorously quantify such a standard extract (exhaustive cleanup of the extract followed by microgravimetric or GC/FID quantification) are described in Sections 7.3.5 and 7.3.6.

Table 7.3.6

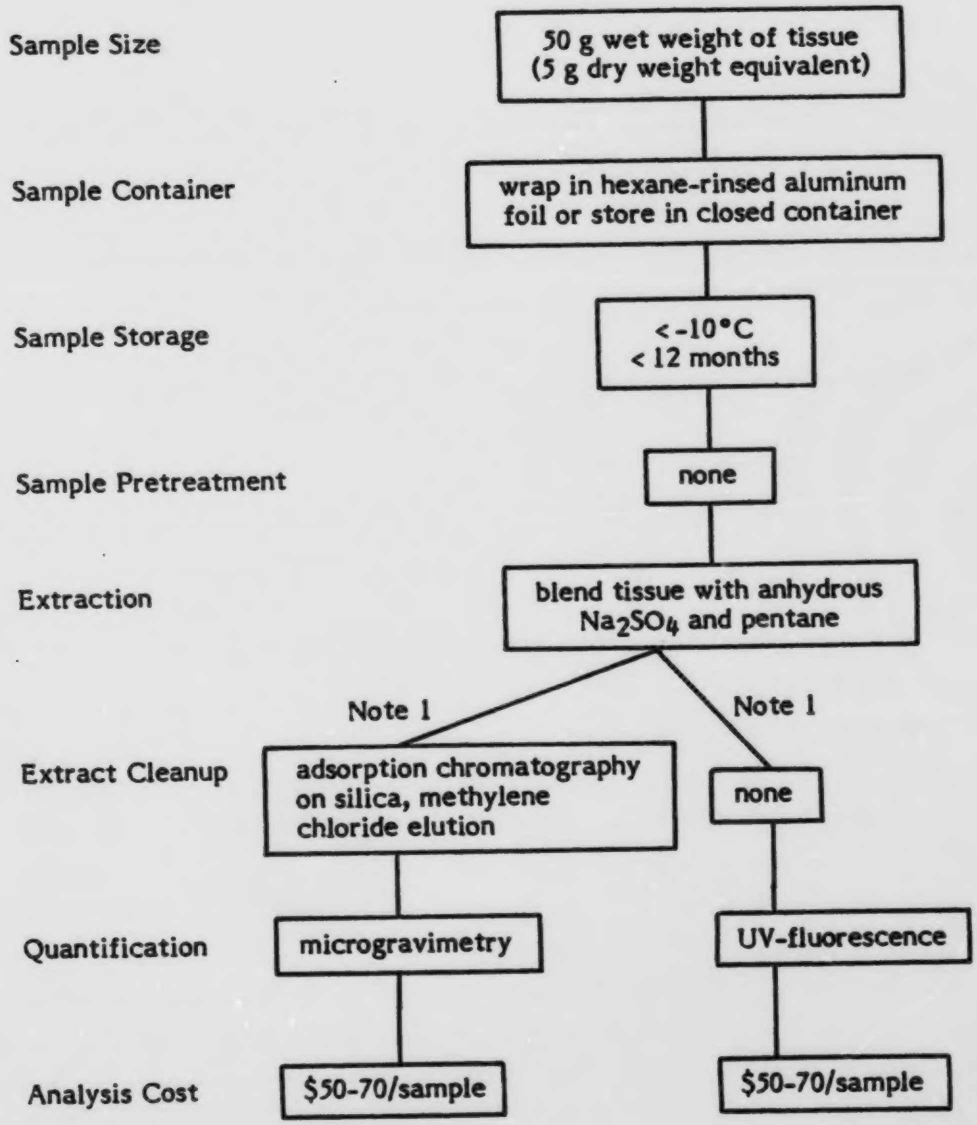
Low Accuracy Determination of Petroleum Hydrocarbons in Sediments


Sample Size
50 g wet weight
(25 g dry weight equivalent)
Sample Container
precleaned glass bottles
(detergent wash, acetone rinse, heat
at 350°C for 16 hours) with screw-on
lid with foil lining
Sample Storage
< -10°C
< 12 months
Sample Pretreatment
air-dry
Extraction
sonication in non-fluorescing
solvent e.g. hexane, cyclohexane
Note 1
Note 1
Extract Cleanup
treatment with
activated copper
adsorption chromatography
on silica, methylene
chloride elution
none
Quantification
microgravimetry
UV-fluorescence
Analysis Cost
$50-70/sample
$50-70/sample

Note 1: The UV-fluorescence method is more specific for petroleum hydrocarbons than the microgravimetric method but use of the microgravimetric method avoids the calibration problems associated with UV-fluorescence method.

Table 7.3.7

Low Accuracy Determination of Petroleum Hydrocarbons in Biota.
Estimation of Contamination Levels


Sample Size
50 g wet weight of tissue (5 g dry weight equivalent)
Sample Container
wrap in hexane-rinsed aluminum foil or store in closed container
Sample Storage
< -10°C
< 12 months
Sample Pretreatment
none
Extraction
blend tissue with anhydrous Na_2SO_4 and pentane
Note 1
Note 1
Extract Cleanup
adsorption chromatography on silica, methylene chloride elution
none
Quantification
microgravimetry
UV-fluorescence
Analysis Cost
$50-70/sample
$50-70/sample

Note 1: The UV-fluorescence method is more specific for petroleum hydrocarbons than the microgravimetric method but use of the microgravimetric method avoids the calibration problems associated with UV-fluorescence method.

7.3.4.2 Microgravimetric Determination

As with methods employing a UV-fluorescence determination of the petroleum hydrocarbons, a rapid and inexpensive extraction technique is selected when microgravimetric quantification is employed. However, in this case cleanup of the extract solution is required to ensure some specificity of the analysis for petroleum hydrocarbons. This increases the analysis time and cost slightly but avoids the problems associated with calibration of the UV-fluorescence response. The microgravimetric method is also susceptible to error due to loss of volatile petroleum components or contamination of the sample by dust, and it is less specific towards petroleum than UV-fluorescence. The use of microgravimetry is described by Clark and Brown (1977).

7.3.4.3 Sediment Extraction and Extract Cleanup

It is recommended that pre-dried sediment be extracted by sonication in cyclohexane (Tan, 1979) when a low accuracy analysis is being carried out. The rationale for selection of this method is discussed in Section 7.3.3.2.

UV-fluorescence measurements can be obtained directly on this extract solution. If greater specificity in the UV-fluorescence measurement is desired, or if the petroleum hydrocarbons are determined by microgravimetry, some prior cleanup of the extract is required.

A cleanup technique is required to separate co-extracted polar lipid material from the petroleum hydrocarbons. Although several techniques have been used for this purpose, adsorption chromatography on a silica column using methylene chloride:pentane for elution is recommended (Tan, 1979). It is a rapid and inexpensive technique and provides reasonable cleanup of extract solutions with good recovery of petroleum hydrocarbons. Loss of some petroleum hydrocarbons can be experienced if alumina or florisil columns are used and they should be avoided in quantitative analysis. The use of a gel column such as Sephadex LH20 will provide only a partial cleanup of the extract (it is normally used in addition to silica cleanup to separate lipids and provide fractionation of the petroleum into non-polar and aromatic fractions. See Section 7.3.8).

Elemental sulfur must be removed from sediment extracts prior to microgravimetric determinations. This is accomplished by treatment of the extract solution with activated copper powder (Blumer, 1957). This treatment is not required prior to UV-fluorescence measurement (or for biota extracts).

7.3.4.4 Biota Extraction and Extract Cleanup

It is recommended that biota tissue be extracted by blending with pentane and anhydrous sodium sulfate (Gay et al., 1980). The rationale for selection of this method is discussed above in Section 7.3.3.3.

UV-fluorescence measurements are made directly on this extract solution. Cleanup of the extract is required prior to microgravimetric determinations. As discussed above for sediment extracts (Section 7.3.4.3) adsorption chromatography on silica using methylene chloride:pentane for elution is the recommended method (Tan, 1979). No treatment for sulfur removal is required.

7.3.5 Quantitative Analysis Procedures Providing Moderate Accuracy

Analytical methods of at least moderate accuracy are required to monitor more subtle changes in petroleum contamination levels in sediment and biota samples. Suitable methods are those which are relatively insensitive to variations in petroleum composition. In certain situations, the added requirement of low detection limit may be necessary. The recommended analysis procedure for sediment samples is outlined in Table 7.3.8 and the procedure for biota in Table 7.3.9.

7.3.5.1 Determination of Petroleum Hydrocarbons

As indicated in Table 7.3.1, several techniques are sufficiently accurate to monitor trends in petroleum concentrations in extract solutions. These are ultraviolet spectroscopy and UV-fluorescence, microgravimetry and gas chromatography with either flame ionization or mass spectral detection. Both the ultraviolet and the UV-fluorescence responses are difficult to calibrate accurately and neither technique is recommended for use when the petroleum composition is variable due to fractionation or degradation (see discussion in Section 7.3.4.1 and in Sections III-3.4.6 of Volume 1). Therefore either a gas chromatographic or a microgravimetric quantitation step is preferred in this situation. These two techniques are comparable in accuracy but microgravimetry is less sensitive than either GC/FID or GC/MS. However, microgravimetry is less expensive and is recommended for use when its higher detection limit (10 $\mu g \cdot g^{-1}$) is acceptable. A GC method is recommended when lower detection limits (1 $\mu g \cdot g^{-1}$) are required. GC/FID

Table 7.3.8

Moderate Accuracy Determination of Petroleum Hydrocarbons in Sediments

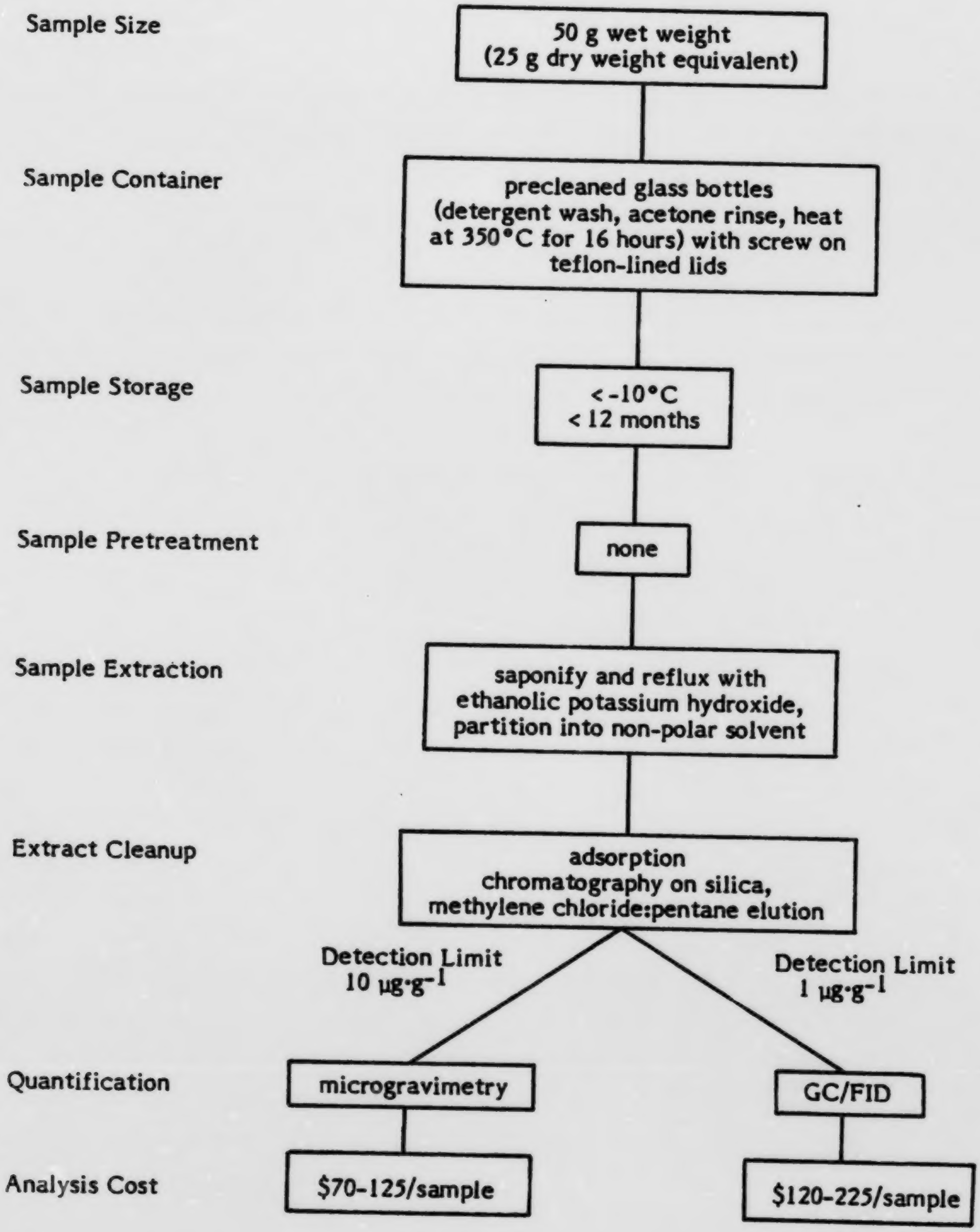
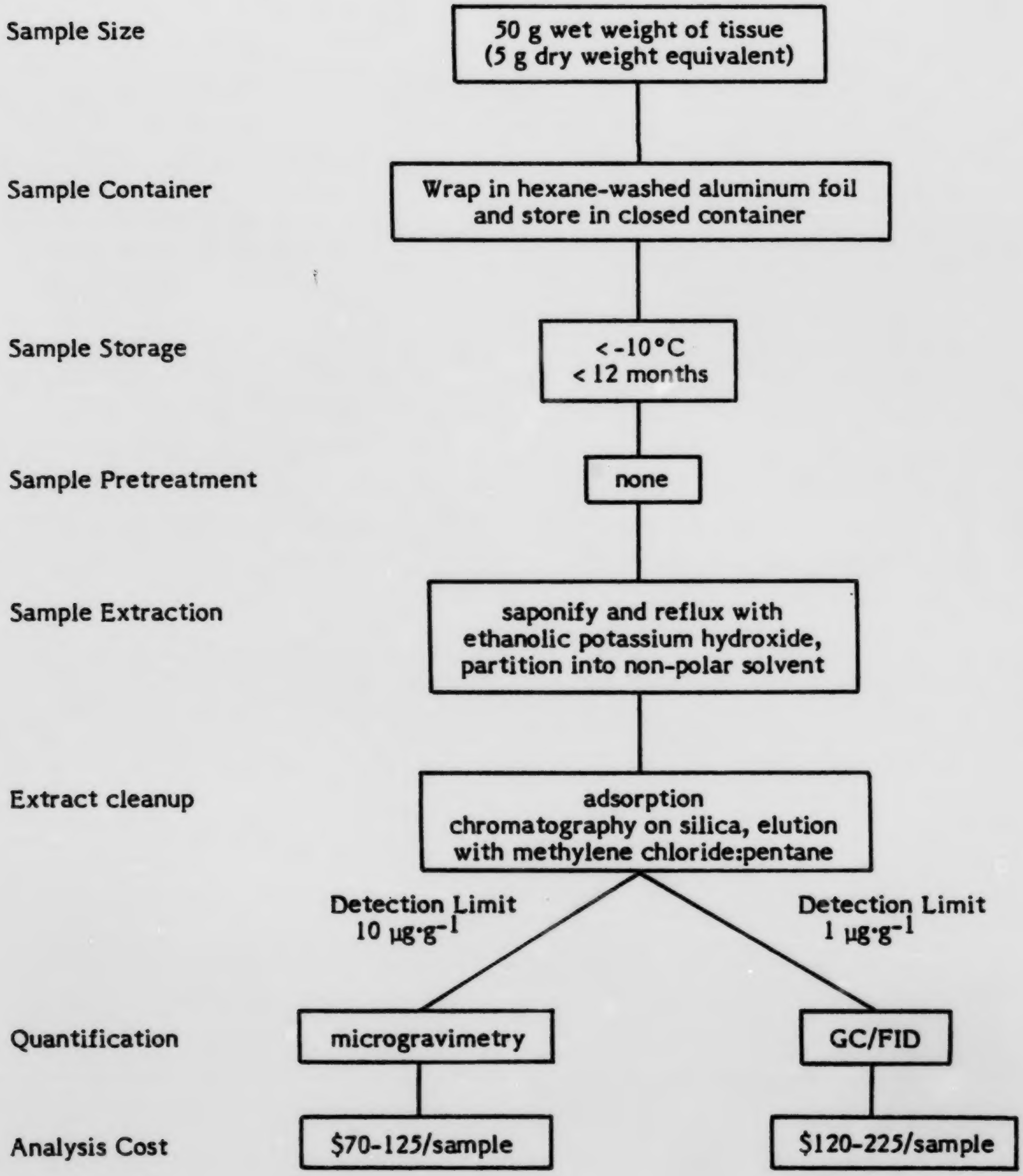

Sample Size
50 g wet weight
(25 g dry weight equivalent)
Sample Container
precleaned glass bottles
(detergent wash, acetone rinse, heat at 350°C for 16 hours) with screw on teflon-lined lids
Sample Storage
< -10°C
< 12 months
Sample Pretreatment
none
Sample Extraction
saponify and reflux with ethanolic potassium hydroxide, partition into non-polar solvent
Extract Cleanup
adsorption chromatography on silica, methylene chloride:pentane elution
Detection Limit 10 µg·g⁻¹
Detection Limit 1 µg·g⁻¹
Quantification
microgravimetry
GC/FID
Analysis Cost
$70-125/sample
$120-225/sample

Table 7.3.9

Moderate Accuracy Determination of Petroleum Hydrocarbons in Biota


Sample Size
50 g wet weight of tissue (5 g dry weight equivalent)
Sample Container
Wrap in hexane-washed aluminum foil and store in closed container
Sample Storage
< -10°C
< 12 months
Sample Pretreatment
none
Sample Extraction
saponify and reflux with ethanolic potassium hydroxide, partition into non-polar solvent
Extract cleanup
adsorption chromatography on silica, elution with methylene chloride:pentane
Detection Limit 10 µg·g-1
Detection Limit 1 µg·g-1
Quantification
microgravimetry
GC/FID
Analysis Cost
$70-125/sample
$120-225/sample

is the technique of choice in this case as it is less expensive, more available and easier to calibrate for quantitative work than GC/MS.

7.3.5.2 Sediment Extraction and Extract Cleanup

When monitoring changes in petroleum contamination levels in samples, an efficient extraction method yielding precise results is required. Although many of the extraction procedures described in Section III-3.4.4.1 of Volume 1 exhibit high recovery of petroleum hydrocarbons, the best precision has been reported when the sediment was saponified before extraction (Wong and Williams, 1980). This procedure (reflux with ethanolic potassium hydroxide, partition into a non-polar solvent) is described in detail by Plumb (1981) and Cretney et al. (1980) and is recommended for accurate quantitative analysis. It has the additional advantages of: 1) not requiring pre-dried sediments, 2) resulting in partially cleaned extract solutions, and 3) eliminating the need for a subsequent sulfur removal step.

Cleanup of the extract solution is required prior to quantification of the petroleum hydrocarbons. Silica adsorption chromatography and gel permeation chromatography on Sephadex LH20 both provide effective clean-up methods. For total hydrocarbon measurements, with no fractionation of hydrocarbons, a silica column eluted with methylene chloride:pentane will provide an adequate cleanup (Tan, 1979). For compound separation into non-polar and aromatic fractions, a compound method using Sephadex LH20 and silica columns is recommended (Giger and Schaffner, 1978; see Section 7.3.6). Low recoveries of some aromatics can occur with alumina or florisil columns. Their use is not recommended therefore for quantitative analyses.

7.3.5.3 Biota Extraction and Extract Cleanup

Saponification of the tissue by reflux with ethanolic potassium hydroxide, followed by partition of the hydrocarbons into a non polar solvent (Cretney et al., 1980) is the recommended extraction technique for this analysis. No prior drying or other treatment of the sample is then required. Although saponification is a slightly less efficient extraction technique than a soxhlet extraction, it is more rapid and the accompanying solvent partition step eliminates much of the polar lipid material which could interfere in the subsequent quantification step (see discussion in Section III-3.4.4.2 of Volume 1).

The cleanup, fractionation and quantification steps of biota extracts produced in this manner are identical to those required for sediment extracts. As discussed in Section 7.3.5.2, adsorption chromatography on silica columns is the most appropriate cleanup procedure when determining the total hydrocarbon content of extract solutions. Fractionation of the cleaned extract is not carried out unless compositional information is also required. In such a case the aliphatic and aromatic fractions of the extract obtained from Sephadex LH20 columns (see Section 7.3.8) can be cleaned and quantified separately.

7.3.6 Quantitative Analysis Procedures Providing High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons

An analytical method of high accuracy and high sensitivity is required to monitor baseline levels of petroleum hydrocarbons in sediment and biota samples. The utmost care is required in sample handling and the most rigorous extraction and extract cleanup procedures are carried out. The most accurate and sensitive quantification technique is used. The recommended analysis procedure for sediment samples is outlined in Table 7.3.10 and the procedure for biota in Table 7.3.11.

7.3.6.1 Determination of Petroleum Hydrocarbons

As indicated in Table 7.3.1, only gas chromatographic techniques (either GC/FID or GC/MS) have sufficient sensitivity to accurately measure the low levels of petroleum hydrocarbons expected in arctic background samples. A detection limit of 1 $\mu g \cdot g^{-1}$ can be achieved. The microgravimetric technique is also accurate but is useful only for samples containing higher levels of petroleum contamination (> 10 $\mu g \cdot g^{-1}$). GC/FID is recommended for quantification of petroleum hydrocarbons as it is less expensive, more available and easier to calibrate for quantitative work (Farrington and Tripp, 1975). However, GC/MS can be used if compositional information is also required (see Section 7.3.8) and is preferred for the analysis of polycyclic aromatic hydrocarbons (Tan, 1979; Shaw, 1979).

7.3.6.2 Sediment Extraction and Extract Cleanup

A rigorous extraction procedure is required to completely extract petroleum hydrocarbons from sediment. Saponification of the sediment sample (by reflux in

Table 7.3.10

High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons in Sediments

Sample Size	50 g wet weight (25 g dry weight equivalent)
Sample Container	precleanead glass bottle (detergent wash, acetone rinse, heat at 350°C for 16 hours) with teflon-lined screw-on lid
Sample Storage	< -10°C < 6 months
Smaple Pretreatment	none
Sample Extraction	saponify and reflux with ethanolic potassium hydroxide, partition into non-polar solvent
Sample Cleanup	gel permeation chromatography on Sephadex LH20
	adsorption chromatography on silica, methylene chloride:pentane elution
Quantification	GC/FID
Analysis Cost	$275-400/sample

Table 7.3.11

High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons in Biota

Sample Size	50 g wet weight of tissue (5 g dry weight equivalent)
Sample Container	wrap tightly in hexane-washed aluminum foil and store in closed container
Sample Pretreatment	none
Sample Extraction	saponify and reflux with ethanolic potassium hydroxide, partition into non-polar solvent
Extract Cleanup	gel permeation chromatography on Sephadex LH20
	adsorption chromatography on silica, methylene chloride:pentane elution
Quantification	GC/FID
Analysis Cost	$275-400/sample

ethanolic potassium hydroxide and partitioning into a non-polar solvent) is reported to result in high recoveries of petroleum hydrocarbons, with good precision and minimal interference from polar co-extracted material. This is the recommended extraction procedure - it is described by Plumb (1981) and by Cretney et al. (1980). Other extraction procedures may result in incomplete recovery of petroleum hydrocarbons (e.g., ball mill tumbling methods) or produce extracts that include high levels of co-extracted material and require a more thorough cleanup (e.g., soxhlet methods).

Cleanup of the extract is required prior to the quantification step to remove interfering co-extracted material. The choice of extract clean up and separation method depends on the intended goal and the instrumental technique to be used. However, for the analysis of petroleum hydrocarbons at baseline levels, the fossil fuel hydrocarbons must be separated from interfering hydrocarbons such as those of recent biological origin and higher molecular weight lipids. This is desirable even if the technique normally does not require any type of separation, such as a total hydrocarbon determination by gas chromatography. The method of choice for this situation is gel permeation chromatography on Sephadex LH20 followed by silica chromatography (Giger and Schaffner, 1978). Adsorption chromatography on alumina or Florisil may result in loss of petroleum hydrocarbons and should be avoided.

7.3.6.3 Biota Extraction and Extract Cleanup

Extraction of biota tissue by saponification (reflux in ethanolic potassium hydroxide, partitioning into a non-polar solvent (Cretney et al., 1980)) is the recommended procedure. No prior drying or other treatment of the sample is then required. Although saponification is a slightly less efficient extraction technique than a soxhlet extraction, it is more rapid and the resultant extract contains fewer potential interferences to the quantification step.

The recommended cleanup procedure is that of Giger and Schaffner (1978) - size separation by gel permeation chromatography on Sephadex LH20 followed by polarity separation by adsorption chromatography on silica. The rationale for this selection is given in Section 7.3.5.2 above.

7.3.7 Quantitative Analysis Procedures for the Determination of Low Molecular Weight Petroleum Hydrocarbons in Sediment and Biota

Because of their volatility, low molecular weight hydrocarbon compounds (those containing less than approximately eleven carbon atoms) in sediment and biota are not amenable to analysis by conventional solvent extraction techniques. However, specialized technqiues based on gas chromatography are available to isolate and quantitate these compounds. Either static headspace sampling or dynamic headspace purging of the sample can be used, followed by a gas chromatographic identification and quantification of the hydrocarbons. The static headspace technique requires no special equipment and is therefore less expensive and more available than the dynamic headspace purge. However, the static technique is an equilibrium technique (the extraction is not taken to completion) and is potentially subject to many sources of error even when elaborate calibration is carried out. Use of this technqiue should be restricted to situations in which only semi-quantitative results are required.

The dynamic headspace technique (Michael et al., 1980) is potentially more accurate as it causes the equilibrium to be pushed toward completion resulting in almost complete extraction of the volatile hydrocarbon components. However a special apparatus is required, thus limiting the availability of the analysis. This is a recommended procedure, however, if quantitative results are required.

7.3.8 Qualitative Analysis of Petroleum Hydrocarbons in Sediment and Biota Samples (Compositional Information)

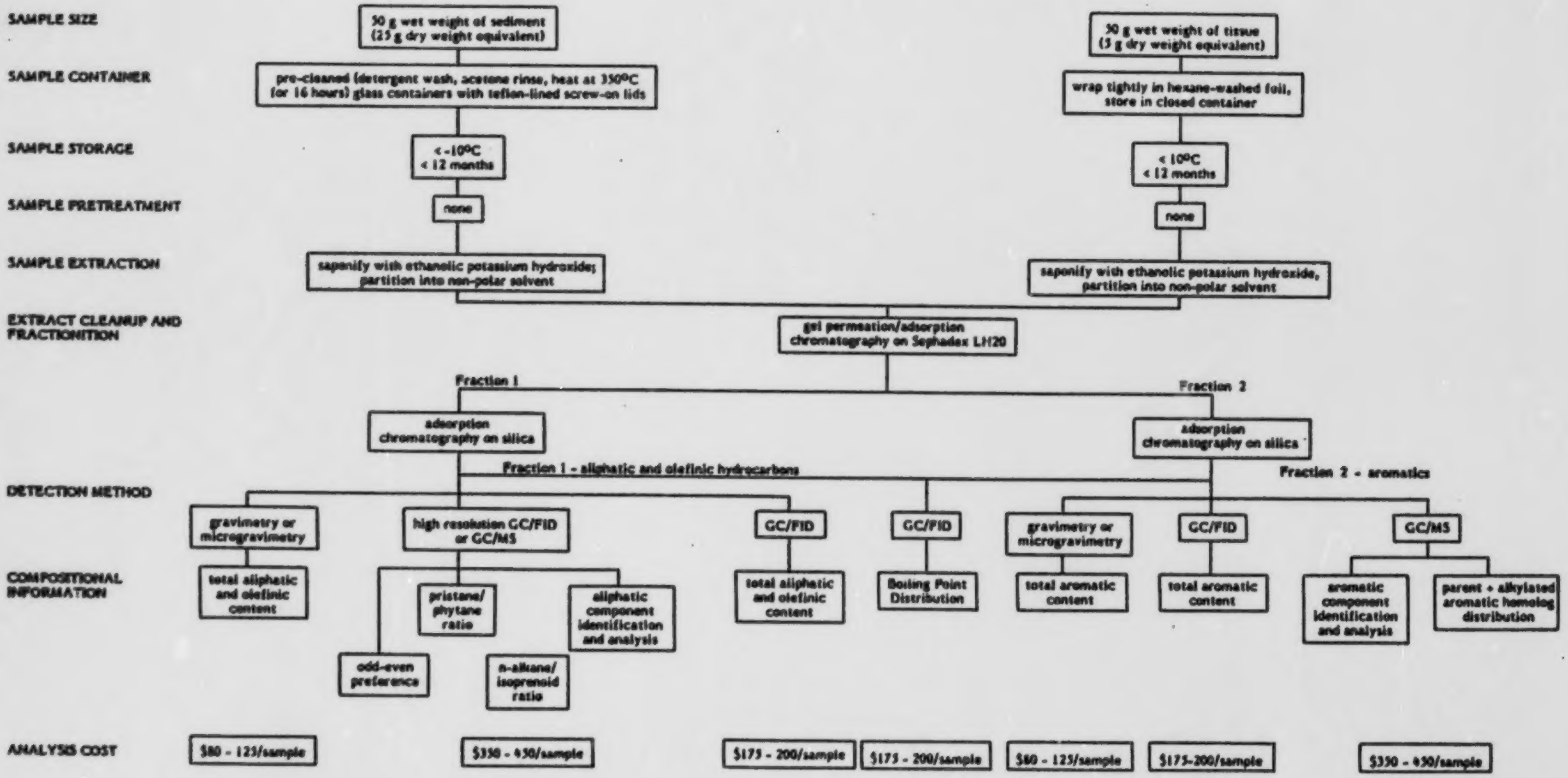
The types of compositional information which can be determined on the petroleum hydrocarbons extracted from sediment and biota samples are listed in Table 7.3.3. For these analyses, careful sample handling is required to avoid loss of hydrocarbon components or contamination of the sample. An efficient extraction followed by rigorous cleanup and fractionation of the extract is required. The procedures for compositional analysis of sediments and biota tissue are outlined in Table 7.3.12.

The saponification extraction recommended above for sediment and tissue (see Sections 7.3.6.2 and 7.3.6.3) is also useful here. This method provides efficient recovery of petroleum hydrocarbons from both sediment and biota samples while yielding moderately clean extract solutions that do not cause interference in the instrumental analysis.

Extract cleanup and fractionation are required prior to compositional analyses of the extracted petroleum. Adsorption chromatography on Florisil or on silica or silica/alumina columns and adsorption/size separation on Sephadex LH20 are commonly used. Low recoveries of some aromatics can be experienced with Florisil

Table 7.3.12

Compositional Analysis of Petroleum Hydrocarbons in Sediments and Biota


SAMPLE SIZE
SAMPLE CONTAINER
SAMPLE STORAGE
SAMPLE PRETREATMENT
SAMPLE EXTRACTION
EXTRACT CLEANUP AND FRACTIONITION
DETECTION METHOD
COMPOSITIONAL INFORMATION
ANALYSIS COST
50 g wet weight of sediment (25 g dry weight equivalent)
pre-cleaned (detergent wash, acetone rinse, heat at 350°C for 16 hours) glass containers with teflon-lined screw-on lids
< -10°C < 12 months
none
saponify with ethanolic potassium hydroxide; partition into non-polar solvent
50 g wet weight of tissue (5 g dry weight equivalent)
wrap tightly in hexane-washed foil, store in closed container
< 10°C < 12 months
none
saponify with ethanolic potassium hydroxide, partition into non-polar solvent
gel permeation/adsorption chromatography on Sephadex LH20
Fraction 1
Fraction 2
adsorption chromatography on silica
adsorption chromatography on silica
Fraction 1 - aliphatic and olefinic hydrocarbons
Fraction 2 - aromatics
gravimetry or microgravimetry
high resolution GC/FID or GC/MS
GC/FID
GC/FID
gravimetry or microgravimetry
GC/FID
GC/MS
total aliphatic and olefinic content
pristane/phytane ratio
aliphatic component identification and analysis
odd-even preference
n-alkane/isoprenoid ratio
total aliphatic and olefinic content
Boiling Point Distribution
total aromatic content
total aromatic content
aromatic component identification and analysis
parent + alkylated aromatic homolog distribution
$80 - 125/sample
$350 - 450/sample
$175 - 200/sample
$175 - 200/sample
$80 - 125/sample
$175-200/sample
$350 - 450/sample

and alumina columns and they are not recommended for petroleum determinations. Chromatography on silica is required to adequately clean the extract (remove esters) while chromatography on Sepadex LH20 is required to accurately fractionate the sample into aliphatic (non-polar) and aromatic portions. Chemical separation of aromatics by complexation with dimethylsulfoxide or trinitrofluorenone has been used in some methods. Such fractionation is useful when only the concentration of aromatics in the sample is of interest. No other compositional information can be obtained and there does not appear to be any advantage to these methods over Sepadex LH20 fractionation even for simple quantification of the aromatic fraction. **Therefore the cleanup and fractionation procedure of Giger and Schaffner (1978) is recommended whereby the sample is fractionated on a Sepadex LH20 column and the two fractions are cleaned on silica columns to remove non-petroleum contaminants.**

The instrumental technique used for analysis of the aromatic and aliphatic fractions depends on what compositional information is required. GC/FID and GC/MS are most commonly used (Farrington and Tripp, 1975; Shaw, 1979; Tan, 1979). Depending on the goal, quantitative analysis of the aliphatic (non-polar) fraction can be carried out by the techniques recommended in Sections 7.3.4.2, 7.3.5.1 or 7.3.6.1 (gravimetry or gas chromatography). Quantitative analysis of the aromatic fraction can be carried out by the techniques recommended in Sections 7.3.4.1, 7.3.4.2, 7.3.5.1 and 7.3.6.1 above (gravimetry, gas chromatography or UV-fluorescence).

The boiling point distribution (or distillation curve) of the extracted petroleum is also obtained by GC/FID. The aliphatic and aromatic fractions can be recombined prior to this analysis, or analyzed separately.

Several parameters can be determined from the aliphatic fraction of the extract. These include odd-even preference, pristane/phytane ratio and n-alkane/isoprenoid ratio. High resolution (capillary column) GC/FID is required for these analyses. These parameters can also be measured on the unfractionated extract (i.e., before the Sephadex LH20 fractionation) but the results by GC/FID would be less accurate due to a greater possibility of peak overlap.

The aromatic fraction of the extract can be analyzed by GC/MS to provide both a parent aromatic compound distribution and an alkylated aromatic homolog distribution. This information can be obtained by no other technique.

GC/MS is the ideal technique to use for both major and minor aromatic component identification. Major components may be identifiable by high resolution (capillary column) GC/FID, but with less certainty. Major component concentrations can be determined by GC/FID or GC/MS, but minor components must be analyzed by GC/MS.

7.4 Oil and Grease

Oil and grease is a procedurally-defined quantity dependent on the organic solvent and technique used for extraction and on the quantification method employed. Three standard methods exist (Inland Waters Directorate, 1979; EPA (Plumb, 1981) and APHA, AWWA, WPCF, 1980). The various procedures used for oil and grease determinations on sediments are reviewed in Section III-3.5, Volume 1 and discussed below.

7.4.1 Sample Requirements

Approximately 20 g (wet weight) of sediment is required for oil and grease determinations. Precleaned (solvent-rinsed) metal containers or glass jars with teflon-lined lids can be used for sample storage. Sediments are acidified and maintained at -10°C (Inland Waters Directorate, 1979).

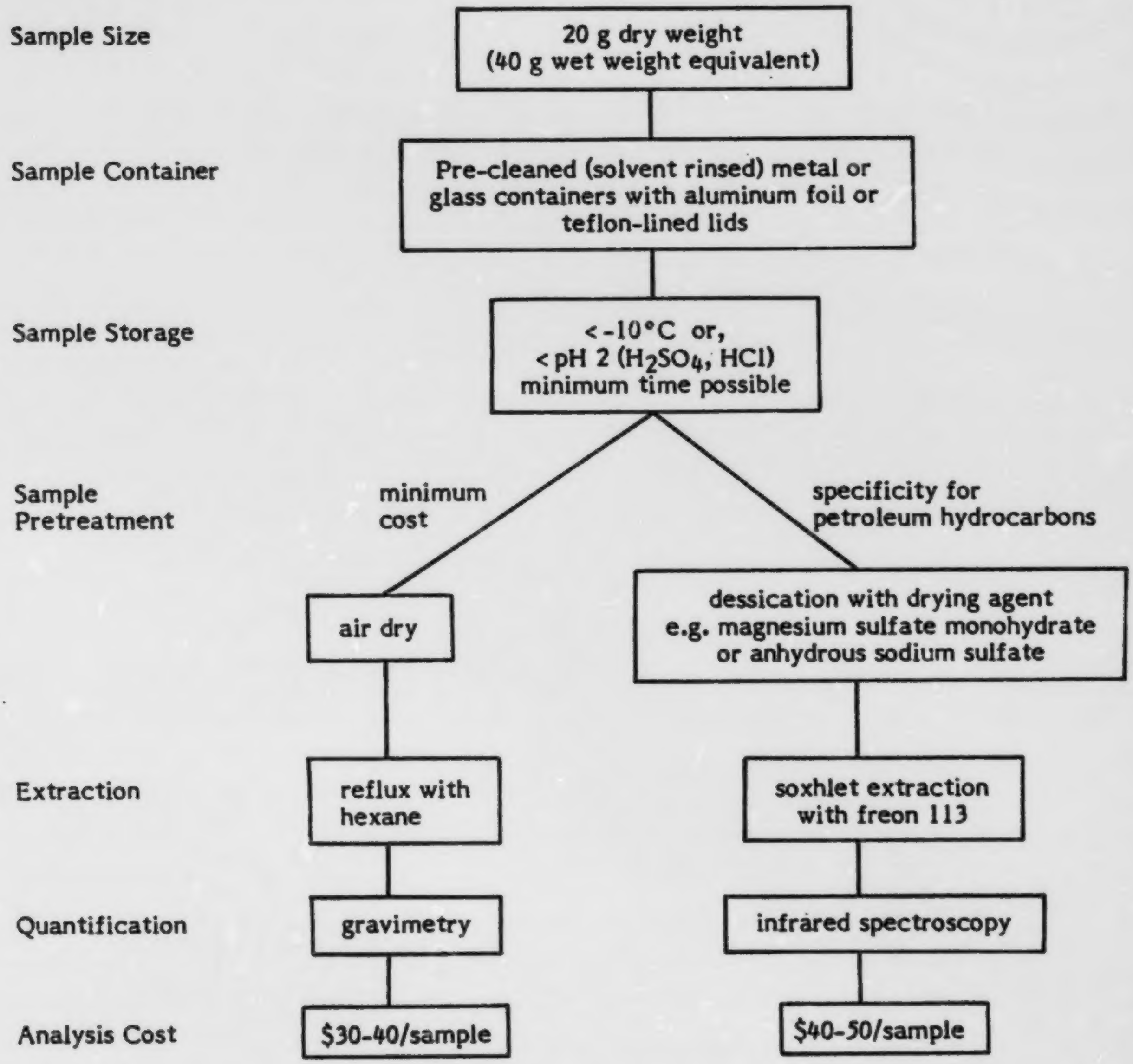
7.4.2 Analysis Procedures

Selection of a method for oil and grease determinations can be based on considerations of cost and accuracy. Variations in the analysis cost will be due essentially to the method chosen for quantification - gravimetry or infrared spectroscopy. A gravimetric (or microgravimetric) based method will be less expensive than an infrared method. However, infrared spectroscopy is more specific to petroleum hydrocarbons while elemental sulfur in the sediment can cause significant error in gravimetric methods. **Therefore a gravimetric based method (Inland Waters Directorate, 1979; Plumb, 1981; APHA, AWWA, WPCF, 1980) is recommended as a low cost method and an infrared method (Plumb, 1981) is recommended as a more petroleum hydrocarbon specific method.** These methods, outlined in Table 7.4.1, will provide comparable detection limits.

For maximum accuracy the sediment should not be air-dried to prevent loss of volatile hydrocarbon material. The sample should be dessicated by grinding with magnesium sulfate or anhydrous sodium sulfate (Plumb, 1981). Soxhlet extraction is recommended in all three standard methods. Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) is used as the extraction solvent as it does not interfere with the infrared quantification. Calibration of the infrared absorbance requires the use of standard solutions and is discussed in detail by Plumb, 1981.

Table 7.4.1

Determination of Oil and Grease in Sediments


Sample Size
20 g dry weight (40 g wet weight equivalent)
Sample Container
Pre-cleaned (solvent rinsed) metal or glass containers with aluminum foil or teflon-lined lids
Sample Storage
< -10°C or, < pH 2 (H_2SO_4, HCl) minimum time possible
Sample Pretreatment
minimum cost
specificity for petroleum hydrocarbons
air dry
dessication with drying agent e.g. magnesium sulfate monohydrate or anhydrous sodium sulfate
Extraction
reflux with hexane
soxhlet extraction with freon 113
Quantification
gravimetry
infrared spectroscopy
Analysis Cost
$30-40/sample
$40-50/sample

A less expensive oil and grease determination, using a gravimetric or microgravimetric measurement of the extractable material, would not require a soxhlet extraction. Reflux extraction of an air-dried sample is often used for speed and convenience (Thomas et al., 1982). Freon (1,1,2-trichloro-1,2,2 trifluoroethane) can be used as the extraction solvent but hexane (Thomas et al., 1982) or petroleum ether (Inland Waters Directorate, 1979) are also suitable. Hexane is commonly used for this purpose and results reported as "Hexane Extractables". The oil and grease or hexane extractables are determined from the weight of non-volatile (room temperature) material in an aliquot of the extract (Inland Waters Directorate, 1979; Plumb, 1981).

7.5 Phenols

The term phenols refers to the range of compounds which have a hydroxyl functional group on an aromatic nucleus. There are two approaches to the determination of environmental phenols: the application of procedurally-defined methods for "total phenols" and the determination of individual compounds. Methods for performing both these types of analyses on sediment and biota samples are reviewed in Section III-3.6 of Volume 1 and discussed below.

7.5.1 Sample Requirements

Approximately 50 g (wet weight) of sediment (Plumb, 1981) or 25 g (wet weight) of tissue (Allen and Sills, 1974) is required for phenols analysis. Sediment samples should be stored in glass containers with teflon-lined lids at 4 C° in the dark (Plumb, 1981). Biota samples should be wrapped tightly in foil and stored in closed containers (Allen and Sills, 1974). The maximum recommended storage time (24 hours; Plumb, 1981) may not be possible for samples collected in the Arctic but analyses for phenols should be carried out as soon as possible. Sediment samples are analyzed wet to minimize loss of phenolic compounds (Plumb, 1981). Biota tissue is pre-dried by homogenization with anhydrous sodium sulfate (Allen and Sills, 1974).

7.5.2 Determination of Total Phenols

Analytical methods for "total phenols" are screening procedures which are sensitive to the general phenol structure. No differentiation among the various phenols is available and all phenolic compounds are reported as "phenol equivalents". Most methods involve a colorimetric quantification step following conversion of the phenolic compounds to a coloured dye complex. Some phenols are non-reactive to this conversion and the molar absorptivity of the dye complex is maximum for phenol itself and may be less for substituted phenols. Thus the results of colorimetric determinations of total phenols represent a minimum estimate. Improved accuracy is obtained if a gas chromatographic quantification is performed (see Section 7.5.2 below on the determination of individual molecules). However "total phenols" is a procedurally dependent quantity and meaningful results can only be achieved if obtained in the context of standardized procedures.

The only standard procedure for total phenols in sediments is that of the EPA (Plumb, 1981) and it is therefore recommended for use. No standard method is available for phenols in biota tissue but it is suggested that a literature method for extracting individual phenol compounds from tissue be used. After cleanup of the tissue extract to remove fats and fat-soluble compounds, the total phenols content can be determined by the EPA procedure for sediment extracts. **The recommended analytical procedures are outlined in Table 7.5.1 for "total phenols" in sediment and in Table 7.5.2 for biota.**

7.5.2.1 Extraction

a) Sediment

In the EPA standard procedure the extraction of phenols from sediments is accomplished by steam distillation (Plumb, 1981). Some literature analyses of specific phenolic compounds recommend a soxhlet extraction of the sediment, however this would result in higher recoveries of the less volatile phenol molecules[1]. Therefore total phenol levels from a soxhlet extraction method would not be comparable to results from the standard EPA methods.

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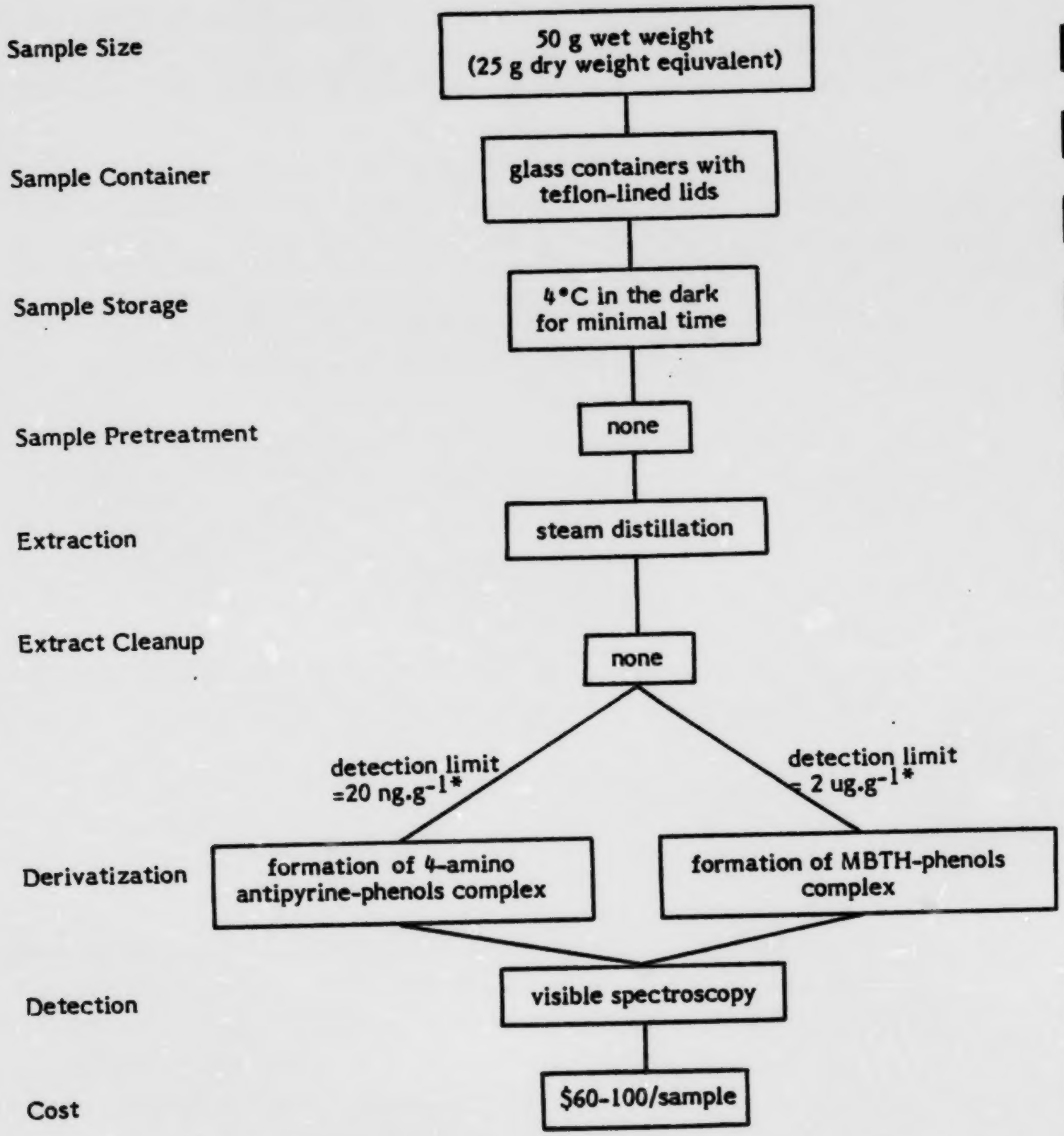
[1] The EPA standard method using steam distillation is adapted from standard methods for water analysis. Water samples (not expected to contain high levels of these less volatile phenols) are not subjected to soxhlet extraction.

Table 7.5.1

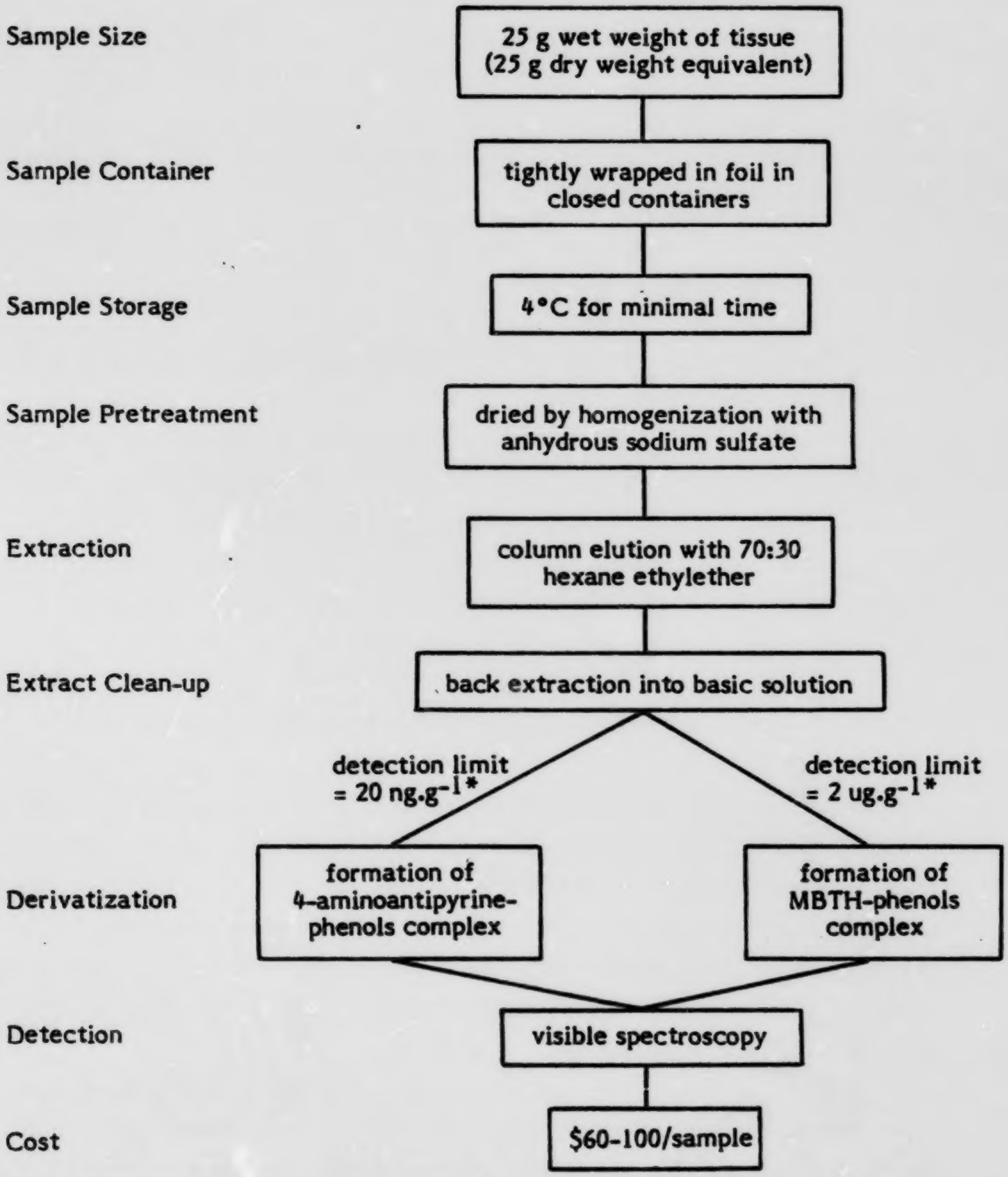
Determination of Total Phenols in Sediments


Sample Size
50 g wet weight (25 g dry weight eqiuvalent)
Sample Container
glass containers with teflon-lined lids
Sample Storage
4°C in the dark for minimal time
Sample Pretreatment
none
Extraction
steam distillation
Extract Cleanup
none
detection limit =20 $ng.g^{-1}$*
detection limit = 2 $ug.g^{-1}$*
Derivatization
formation of 4-amino antipyrine-phenols complex
formation of MBTH-phenols complex
Detection
visible spectroscopy
Cost
$60-100/sample

* Based on 500 mL distillate, 25 g sediment (dry weight)

Table 7.5.2

Determination of Total Phenols in Biota


Sample Size
25 g wet weight of tissue
(25 g dry weight equivalent)
Sample Container
tightly wrapped in foil in
closed containers
Sample Storage
4°C for minimal time
Sample Pretreatment
dried by homogenization with
anhydrous sodium sulfate
Extraction
column elution with 70:30
hexane ethylether
Extract Clean-up
back extraction into basic solution
detection limit
= 20 ng.g-1*
detection limit
= 2 ug.g-1*
Derivatization
formation of
4-aminoantipyrine-
phenols complex
formation of
MBTH-phenols
complex
Detection
visible spectroscopy
Cost
$60-100/sample

*Based on 100 mL distillate, 5 g tissue (dry weight)

b) Biota

The recommended procedure for extracting phenolic compounds from tissue uses a 70:30 hexane:ethyl ether mixture and column elution followed by cleanup via back extraction into basic solution (Allen and Sills, 1974). This method is reported to give excellent extraction recoveries and is simple to perform. A second literature procedure (Hattula et al., 1978) for extraction of phenols from biota does not report recoveries and requires a thin layer chromatographic step to separate the phenols from co-extracted material.

7.5.2.2 Quantification Techniques

The extracted phenols can be determined by spectrofluorimetry, by ultraviolet spectroscopy or by visible spectroscopy (colorimetry) after conversion to a 4-aminoantipyrine or 3-methyl-2-benzothiazolinone hydrazone-HCl (MBTH) dye-complex. The standard EPA method for "total phenols" in sediments uses a colorimetric quantification step. Two procedures are given: the formation and measurement of the 4-aminoantipyrine-phenols complex and the formation and measurement of the MBTH-phenols complex. No criteria are given to choose between the two procedures, but the 4-aminoantipyrene method has a lower detection limit (20 $ng.g^{-1}$ assuming 25 g sediment and 500 mL distillate) than the MBTH method (2 $\mu g.g^{-1}$). Both methods use phenol as the calibration standard and results are reported as phenol equivalents. In both cases the molar absorptivity of the dye complex is maximum for phenol itself and may be less for substituted phenols. In addition, the MBTH method detects phenol compounds which do not react with the 4-aminoantipyrine reagent. Thus "total phenols" by these two methods may not be comparable and will give results that are lower than the concentration of "true" phenols. **It is recommended that the MBTH colorimetric determination be used whenever possible and that the 4-aminoantipyrine colorimetric determination be used only when its lower detection limit is required.**

Fluorimetric and UV methods for the determination of "total phenols" are rapid and do not require derivatization of the phenolic compounds. Their main disadvantage is the low molar absorptivity of most phenols as compared to phenol itself. Thus high levels of substituted phenols could be reported as low levels of phenol equivalents. Fluorimetric and UV measurements are also not specific to the phenol structure: other aromatic compounds (including some petroleum hydrocarbons) would be detected and reported as phenols. Results by either of these methods would not be comparable to results by the standard EPA colorimetric methods[1]. The UV and fluorimetric methods are simpler to perform and less expensive then the derivatization-colorimetric procedures but are useful only when other aromatic contaminants are known to be absent and when results are to be compared only with analyses by the same procedure.

7.5.3 Determination of Individual Phenol Compounds

No standard methods exist for the determination of individual phenol compounds in sediment or biota samples. A few procedures are reported in the literature for specific phenol compounds and each of these employs its own extraction and extract cleanup procedure followed by separation and quantification of the phenols. The recommended procedures for determining individual phenols in sediment and biota are outlined in Table 7.5.3. Methods for the determination of chlorophenols (a subset of the phenols group) are discussed in Section 7.6.

7.5.3.1 Extraction

a) Sediment

The steam distillation method used for the extraction step in "total phenols" determinations on sediment samples does not extract all phenols and is not recommended for individual compound determinations. Three solvent extraction procedures are reported in the literature for specific compounds. **The method of Jungclaus *et al.* (1978) (soxhlet extraction with isopropanol) has been used for the widest range of compounds and is suggested for general use.** The resultant extract solution is analyzed without cleanup. If co-extracted non-polar hydrocarbons (e.g., alkanes) interfere with the determination of the phenols they can be separated by adsorption chromatography on silica (Games and Hites, 1977) but this step should be avoided if possible as it could result in loss of some phenol material on the silica.

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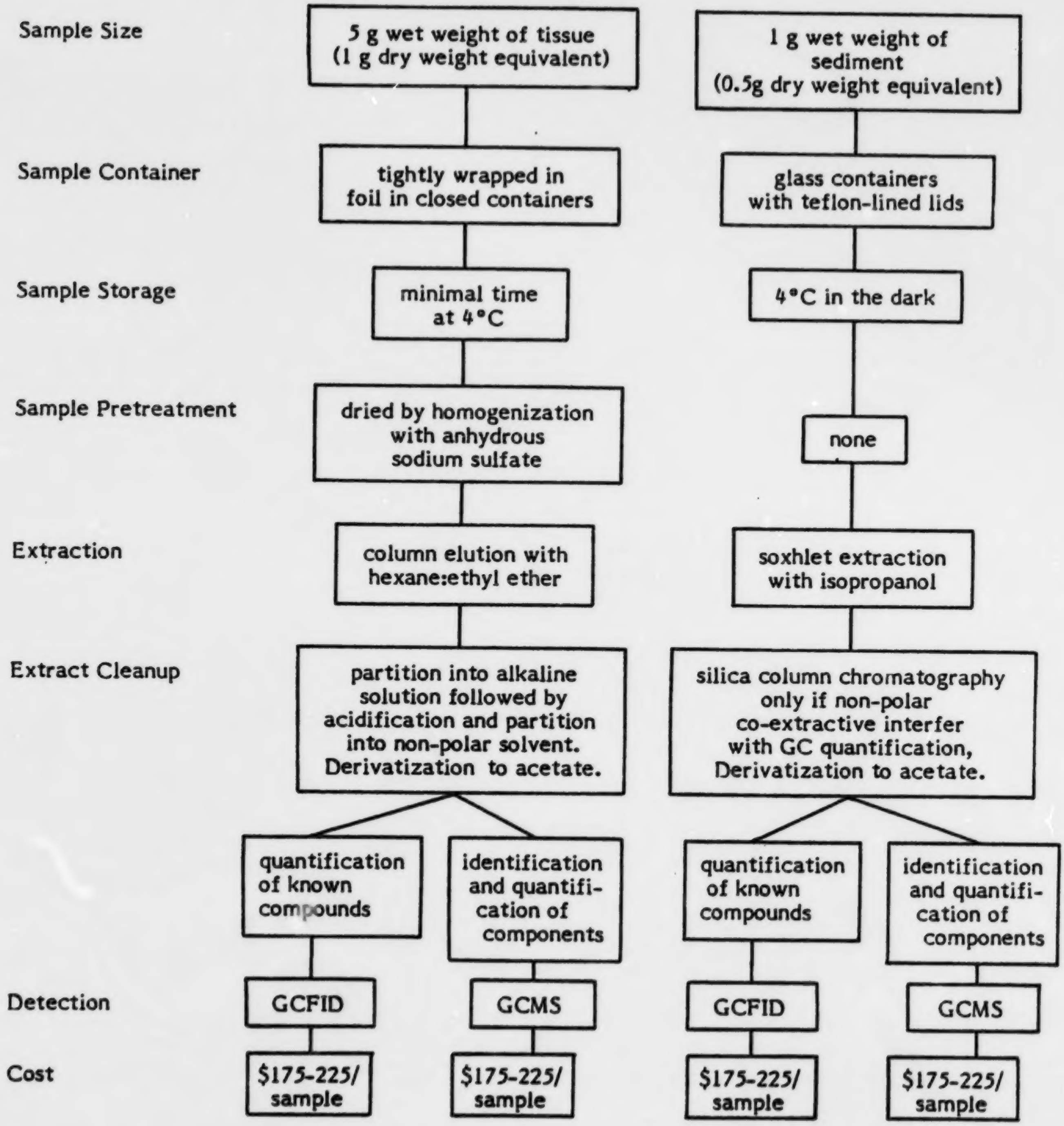
[1] The interpretation of "total phenols" by any of these methods may be difficult.

Table 7.5.3

Determination of Individual Phenolic Molecules


Sample Size
5 g wet weight of tissue (1 g dry weight equivalent)
1 g wet weight of sediment (0.5g dry weight equivalent)
Sample Container
tightly wrapped in foil in closed containers
glass containers with teflon-lined lids
Sample Storage
minimal time at 4°C
4°C in the dark
Sample Pretreatment
dried by homogenization with anhydrous sodium sulfate
none
Extraction
column elution with hexane:ethyl ether
soxhlet extraction with isopropanol
Extract Cleanup
partition into alkaline solution followed by acidification and partition into non-polar solvent. Derivatization to acetate.
silica column chromatography only if non-polar co-extractive interfer with GC quantification, Derivatization to acetate.
quantification of known compounds
identification and quantification of components
quantification of known compounds
identification and quantification of components
Detection
GCFID
GCMS
GCFID
GCMS
Cost
$175-225/ sample
$175-225/ sample
$175-225/ sample
$175-225/ sample

b) Biota

Two solvent extraction procedures are reported for specific phenol compounds in biota tissue. These methods have been applied only to a small number of phenols and it is uncertain how generally applicable they are. **The Allen and Sills (1974) method (extraction by column elution with hexane:ethyl ether) appears more rigorous than that of Hattula et al. (1978) (extraction by shaking with acidic chloroform:diethyl ether) but no convincing evidence can be given to recommend either of these methods over the other.**

Reported cleanup procedures for biota extracts include thin layer chromatography on silica gel and partitioning of the phenols into alkaline solution. The liquid-liquid partition method (Allen and Sills, 1974) is rapid and convenient and should remove co-extracted non-acidic hydrocarbon material while avoiding possible loss of phenols on the silica column.

7.5.3.2 Derivatization and Quantification Techniques

The phenol compounds in extracts are converted to less polar more volatile derivatives and determined by a gas chromatographic technique. **Formation of the acetate derivatives of the phenol molecules is recommended for this purpose (Chau and Coburn, 1974). Derivatization** procedures are discussed in detail in the section on chlorophenols (Section 7.6).

Individual phenols are determined by GC/FID or GC/MS (Coutts et al., 1980). GC/MS can detect lower concentrations and is very powerful for identifying individual components. Chlorophenol compounds can also be determined by GC/EC (see Section 7.6).

7.6 Chlorophenols

Analytical methods for chlorophenols in sediment and biota samples are reviewed in Section III-3.7 of Volume 1. Most methods are individual compound determinations: chlorophenol compounds are separated, identified and quantitated individually. Although the total concentration of chlorophenols can be estimated by summing the level of the individual components identified, this is seldom required. It is more likely that "total phenols" levels are of interest and details of this analysis are discussed in Section 7.5. Methods for determining individual chlorophenols in sediment and biota samples are outlined in Table 7.6.1 and discussed below. The methods recommended are those expected to produce the most accurate and precise results. Coincidentally they are also the fastest and least expensive so that no advantage is gained in using other methods when less accuracy or precision is sufficient.

7.6.1 Sample Requirements

Approximately 10 g (wet weight) of sediment or 25 g (wet weight) of tissue are required for analysis. Careful sample handling is recommended to minimize loss of chlorophenols from the sample. Sediments are stored at -20°C in glass containers with screw-on teflon-lined lids. Glassware and containers are detergent washed, rinsed with acetone and heated at 325°C for 16 hours (EPS, 1979). Biota samples are wrapped in aluminum foil and stored in closed containers at -20°C. No data are available on acceptable storage times but it has been recommended that samples be analyzed as soon after collection as possible. Pretreatment of the samples is not required but air-drying or acidification is used in some methods. Acidification ensures that the chlorophenols are fully protonated and is useful prior to organic solvent extractions. **The effect of drying the sediment and biota samples before analysis is uncertain and is therefore not recommended.**

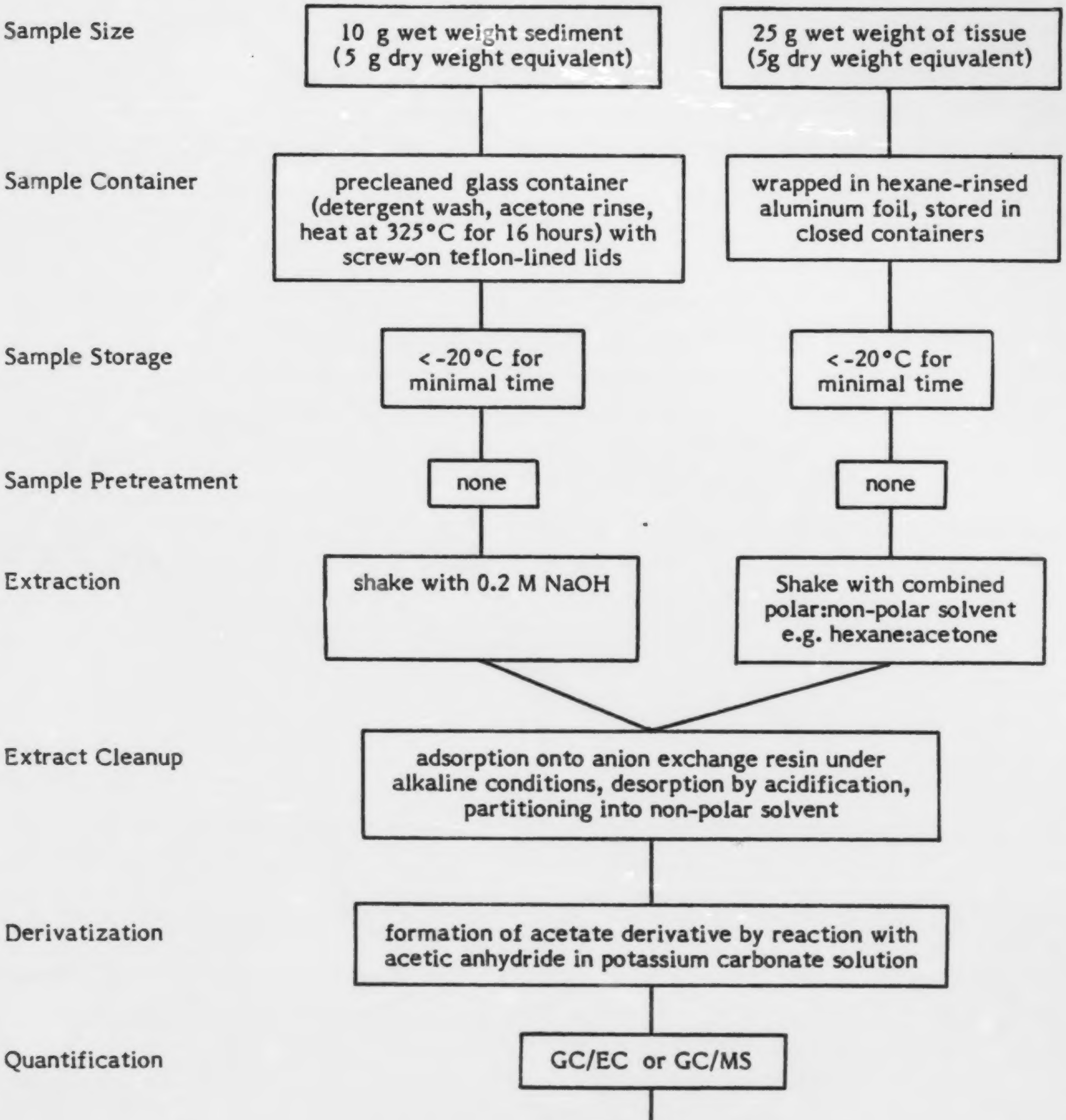
7.6.2 Extraction

a) Sediment

Several sediment extraction procedures are reported in the literature. Some of these report low or variable recoveries[1] of chlorophenols from sediments and these should not be considered for quantitative analysis. Soxhlet extraction with a mixed polar:non-polar solvent gives high recoveries with good precision. **Extraction by shaking with an aqueous sodium hydroxide solution (Renberg, 1974) also results in high recoveries of chlorophenols from sediments and has several advantages compared to soxhlet extraction.** The sodium hydroxide extraction is fast (0.5 hour compared to 24 hours), does not require large volumes of solvent, minimizes loss of volatile chlorophenols and results in cleaner extract solutions which are more readily prepared for gas chromatographic analysis.

Table 7.6.1

Determination of Chlorophenols in Sediment and Biota



Sample Size	10 g wet weight sediment (5 g dry weight equivalent)	25 g wet weight of tissue (5g dry weight eqiuvalent)
Sample Container	precleaned glass container (detergent wash, acetone rinse, heat at 325°C for 16 hours) with screw-on teflon-lined lids	wrapped in hexane-rinsed aluminum foil, stored in closed containers
Sample Storage	< -20°C for minimal time	< -20°C for minimal time
Sample Pretreatment	none	none
Extraction	shake with 0.2 M NaOH	Shake with combined polar:non-polar solvent e.g. hexane:acetone
Extract Cleanup	adsorption onto anion exchange resin under alkaline conditions, desorption by acidification, partitioning into non-polar solvent	
Derivatization	formation of acetate derivative by reaction with acetic anhydride in potassium carbonate solution	
Quantification	GC/EC or GC/MS	
Cost	$175-225/sample	

b) Biota

Reported extraction techniques for tissue are predominantly organic solvent extractions. Non-polar solvents are used if the tissue is pre-dried, combined polar:non-polar solvents are used for wet tissue. The second type of extraction is recommended as pre-drying may cause loss of volatile chlorophenols. **Extraction by shaking with hexane:isopropanol or chloroform:diethyl ether or hexane:acetone followed by hexane:diethyl ether have resulted in high recoveries of chlorophenols.** Such techniques are described by Rudling (1970) and Renberg (1974) as well as others.

7.6.3 Cleanup

Two types of cleanup procedures are reported for sediment and biota extracts. The first of these is liquid-liquid extraction in which the chlorophenols are partitioned from an organic solution into aqueous alkaline solution and then partitioned into fresh organic solvent under acidic conditions. In the second cleanup procedure the chlorophenols are adsorbed from alkaline solution onto anion exchange resin and then partitioned into fresh organic solvent by acidification (Renberg, 1974). Both procedures result in cleaned solutions suitable for gas chromatographic analysis and reasonable recovery rates of chlorophenols. Of the methods **the ion exchange cleanup is more conveniently carried out (fewer manipulations, smaller solvent volumes) and claims slightly higher recovery rates. It is therefore recommended for use on both sediment and biota extracts.**

7.6.4 Derivatization and Quantification

Chlorophenol components are converted to more volatile less polar derivatives for gas chromatographic analysis. Methyl ether, ethyl ether, trimethylsilyl and acetate derivatives have been used. The selection of a derivative is based on convenience and is not critical to the accuracy of the analysis results if correct use is made of calibration solutions and internal standards. Methyl and ethyl ether derivatives (EPS, 1979) are most often employed in the literature but the

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[1] Recovery data was reported for combined extraction-extract cleanup steps. Low reported recoveries may therefore not be due to the extraction technique, but that possibility can not be eliminated.

acetate derivatives (Chau and Coburn, 1974) eliminate interference from any organic acids still in the extract solution. They are therefore recommended for use in chlorophenol determinations. A paper by Chau and Coburn (1974) discuss their preparation in detail.

Either GC/EC or GC/MS can be used for determination of chlorophenol compounds via their volatile derivatives (Landner et al., 1977; Wegman and Van den Broek, 1983). These techniques provide similar detection limits for chlorophenols but GC/MS is more useful for identifying unknown components.

7.7 Chlorinated Hydrocarbons

Analytical methods for the determination of chlorinated hydrocarbon pesticides and PCBs in sediments and biota tissue are reviewed in Section III-3.8 of Volume 1. In these procedures pesticides and PCBs are extracted simultaneously from the samples but are separated prior to their determination by gas chromatography. Pesticides are reported as concentrations of specific individual components or as DDTs (commonly the sum of the concentrations of o,p' and p,p' - isomers of DDT, DDD and DDE). PCB concentrations are reported in terms of technical formulation equivalents, e.g. "Aroclor 1254 equivalents" or occasionally as concentrations of individual PCB compounds.

The Inland Waters Directorate (1979) and the EPA (Plumb, 1981; EPA, 1980) have published standard methods for the analysis of chlorinated hydrocarbons and PCBs in sediment and tissue samples. These methods differ in their extraction and extract cleanup steps but use similar procedures to fractionate and determine the chlorinated hydrocarbons in the extract. EPS (1979) has published a method for determining PCBs in sediment but the method is not applicable to chlorinated hydrocarbon pesticide determinations. Recommended analysis procedures for chlorinated hydrocarbons (PCBs and pesticides) in sediments and biota are outlined in Tables 7.7.1 and 7.7.2 respectively.

7.7.1 Sample Requirements

Approximately 20 g (wet weight) of sediment or 10 g (wet weight) of tissue is required for analysis. Sediment samples should be stored in glass or metal containers. The standard methods recommend either hexane extracted teflon or heavy duty

Table 7.7.1

Determination of Chlorinated Hydrocarbons in Sediments

Sample Size	20 g wet weight (10 g dry weight equivalent)
Sample Container	Precleaned glass container (detergent wash, acetone, ethyl acetate and hexane rinsed, heat at 350° for 16 hours)
Sample Storage	<-20°C for eight weeks
Sample Pretreatment	none
Extraction	Blend with acetonitrile and water
Extract Cleanup and Fractionation	Partition into hexane
	Treatment with activated copper
	Gel permeation chromatography on Biobeads or Sephadex LH20
	Adsorption chromatography on Florisil (four fractions)
	Optional additional treatment with activated copper
Quantification	GC/EC
Verification	GC/MS
Cost	$350-550/sample

Table 7.7.2

Determination of Chlorinated Hydrocarbons in Biota

Sample Size	10 g wet weight of tissue (1-2 g dry weight equivalent)
Sample Container	Wrapped in hexane-rinsed aluminum foil, stored in closed container
Sample Storage	< - 20°C for eight weeks
Sample Pretreatment	none
Extraction	Blend with acetonitrile
Extract Cleanup and Fractionation	Partition into petroleum ether
	Gel permeation chromatography on Biobeads or Sephadex LH20
	Adsorption chromatography on Florisil (4 fractions)
Quantification	GC/EC
Verification	GC/MS
Cost	$375-575/sample

aluminum foil-lined lids for the containers (Plumb, 1981). Glassware and containers should be precleaned by washing in detergent, rinsing with acetone, pesticide-grade ethyl acetate and finally pesticide-grade hexane and heating at 350°C for 16 hours (Plumb, 1981). Biota samples should be wrapped tightly in aluminum foil and stored in closed containers. Other than teflon, **plastic containers and utensils are difficult to clean and are a common source of analytical interference (from plasticers, stabilizers, fillers, etc.), and should therefore be avoided during all stages of the analysis.** Sediment and biota samples should be stored frozen (-20°C) to minimize microbial degradation of the chlorinated hydrocarbons. Samples can be stored under these conditions for at least eight weeks but a maximum storage time has not been reported.

No pretreatment of either sediment or biota samples is required. Some literature methods call for air-drying, oven-drying, or freeze-drying of the sample before extraction but this may lead to loss of volatile chlorinated hydrocarbons. Immediately prior to analysis free water can be removed from the thawed sediments by decantation or centrifugation (Plumb, 1981; Inland Waters Directorate, 1979; Goerlitz and Law, 1974) but thawed tissue samples are analyzed without loss of fluid (Oliver and Bothen, 1982).

7.7.2 Extraction

a) Sediments

A variety of extraction techniques and solvent systems have been used for analysis of chlorinated hydrocarbons in sediments (see Table III-3.28 in Volume 1). Soxhlet extraction has been reported to result in the highest recovery of chlorinated hydrocarbons but also extracts a large amount of lipid material making extract cleanup more difficult. The EPS (1979) procedure for determination of PCBs uses a soxhlet extraction but follows it with a harsh cleanup treatment (saponification with ethanolic potassium hydroxide and oxidation with chromium trioxide) that destroys certain chlorinated hydrocarbon pesticides.

The EPA (Plumb, 1981) and Inland Waters Directorate (1979) methods extract chlorinated hydrocarbons from sediment by blending the sample with acetonitrile in the presence of water. This method provides a compromise between efficient recovery of chlorinated hydrocarbons and cleanability of the resultant extract solution. An alternate EPA procedure (Plumb, 1981) is also reported: partially dried sediment is extracted by column elution with 1:1 hexane:acetone. No comparison of the two EPA procedures is given, nor any criteria for choosing between them, so it is assumed that they produce comparable results. The acetonitrile/blending method is more like the extraction procedures used for biota (discussed below), thus is more convenient when both types of samples are being analyzed. Other extraction procedures (e.g., sonication) could likely produce similar results but **the acetonitrile/blending method is published as a standard procedure in use in Canadian laboratories (Inland Waters Directorate, 1979) and is recommended on that basis.** No saving in time or expense is to be gained by selecting one of the alternate extraction techniques discussed in Section III-3.8.4 of Volume 1.

b) Biota

A variety of extraction techniques and solvent systems have been used for the analysis of chlorinated hydrocarbons in biota (see Section III-3.8.4.2 of Volume 1). All of these methods are reported to result in efficient recovery of chlorinated hydrocarbons from tissue samples and there is no clear reason to select one method over the others. The EPS method (1979) for PCBs in biota calls for soxhlet extraction of the tissue, but as for sediments, this results in large quantities of co-extracted material which complicate extract cleanup and fractionation. The EPS cleanup procedure, which selectively destroys certain chlorinated hydrocarbon pesticides, is not applicable to the determination of all chlorinated hydrocarbons. The Inland Waters Directorate (1979) and the EPA (1980) methods use less drastic extraction procedures. The tissue sample is blended with acetonitrile to extract the chlorinated hydrocarbons (Inland Waters Directorate, 1979) or with petroleum ether to extract the fat from the tissue; the fat is subsequently partitioned with acetonitrile to extract the chlorinated hydrocarbons (EPA, 1980). **The Inland Waters Directorate method is recommended for analysis of chlorinated hydrocarbons in biota tissue.** It is the same procedure recommended above for sediment samples and is therefore conveniently applied to biota. The EPA method was designed for use on all kinds of tissue samples, which likely explains the extra petroleum ether extraction step.

7.7.3 Extract Cleanup and Fractionation

The purpose of the extract cleanup and fractionation steps is to remove co-extracted interferences (such as lipids and elemental sulfur) from the extract and to fractionate the chlorinated hydrocarbons into groups suitable for gas chromatographic analysis. Combinations of liquid-liquid partitioning, carbon adsorption, gel permeation chromatography and adsorption chromatography on silica, alumina and Florisil have been used.

Liquid-liquid partitioning is recommended as a rapid and effective method for the preliminary cleanup of the chlorinated hydrocarbons from co-extracted polar lipids. This procedure is particularly attractive when acetonitrile has been used as an extraction solvent as it is immiscible with saturated hydrocarbon solvents (e.g., pentane, hexane) into which the chlorinated hydrocarbons can be readily partitioned.

Extract cleanup by partitioning the chlorinated hydrocarbons from the acetonitrile extract solution into petroleum ether is described in the Inland Waters Directorate (1979) standard method and is recommended for use. Additional cleanup procedures (adsorption chromatography, gel permeation chromatography, and carbon adsorption) are desirable for heavily contaminated sediment extracts or biota. As adsorption chromatography on silica or alumina may result in some loss of chlorinated hydrocarbon components, **gel permeation chromatography on Biobeads SX-3 or Sephadex LH20 is recommended for additional cleanup.** It is the least reactive cleanup material, gives high recoveries and can be automated. Such a procedure has been described by the EPA (1980) for the cleanup of biota extracts for chlorinated hydrocarbon analysis.

Adsorption chromatography on Florisil columns is the recommended method of separating chlorinated hydrocarbons into groups suitable for GC analysis. This method is well characterized in the literature and is used in all the standard methods. It is described in detail by Plumb (1981) and in the Inland Waters Directorate method (1979). Four fractions are obtained from the Florisil column and each is analyzed separately. A listing of chlorinated hydrocarbons found in each fraction is given by Plumb (1981) and in the Inland Waters Directorate method (1979). If PCBs and/or a complex mixture of chlorinated hydrocarbon pesticides are present in the sample, the extract must be fractionated on the Florisil column to ensure that chromatographic peaks due to the individual components can be resolved.

Elemental sulfur must be removed from sediment extracts prior to quantification by gas chromatography (discussed below). Treatment of the extract with either mercury or copper has been shown to effectively remove the sulfur interference with no effect on chlorinated hydrocarbons in the solution. **Treatment with activated copper (Plumb, 1981) is the recommended method** as it is less hazardous and generally requires only one application (repetitions of the mercury treatment may be required).

7.7.4 Quantification

Gas chromatography with either electron capture (GC/EC) or mass spectral detection with selected ion monitoring (GC/MS/SIM) is used for quantification of chlorinated hydrocarbon pesticides and PCBs (Ernst et al., 1976; Castelli et al., 1983; Plumb, 1981; EPS, 1979). These techniques are extremely sensitive for chlorinated compounds and detection limits of approximately 0.1 $ng \cdot g^{-1}$ can be obtained for both sediment and tissue samples. Both GC/MS and GC/EC provide rapid, reliable quantification of chlorinated hydrocarbons and the choice of technique should be based upon cost, availability and the selectivity required. GC/EC is more common and less expensive. GC/MS, although less commonly used, is more selective and can, at a lower sensitivity, provide full mass spectral confirmation.

a) Chlorinated Hydrocarbon Pesticides

GC/EC and GC/MS/SIM use similar approaches of calibrated instrument response to infer and quantify a target compound. As in any GC method, a single chlorinated hydrocarbon assignment in a GC/EC chromatogram is based upon the detection of a peak within the narrow retention time window observed for the authentic material. An assignment is only considered as an inference if that compound is expected to be present in the appropriate Florisil column fraction. With GC/MS/SIM, additional confirmation is available as several characteristic ions of the analyte may be monitored and only when these ions co-vary and maximize at the appropriate retention time with the same relative ion abundance as the standard, is that compound confirmed.

b) PCBs

The identification of PCBs by GC/EC or GC/MS uses the same criteria as for the single compound GC analyses (Section 7.3.4a) but is complicated by the complex nature of these products. For analyses using GC/EC, the appearance in a chromatogram of the appropriate Florisil fraction (fraction 1) of peaks within the retention time windows as found in an authentic PCB calibration standard and exhibiting a similar distribution of GC peak intensities, is taken as an inference of that PCB formulation. The alteration of PCB composition in the environment by multiple sources, physical fractionation, degradation and photolysis will result in an environmental PCB extract that will not correspond to any particular commercial PCB formulation. Several approaches have been used to resolve this problem, the most common being quantification as a commonly encountered PCB formulation, quantification as individual components or by perchlorination of the PCB to decachlorobiphenyl and its analysis as a single compound. Analysis of individual components is difficult by GC/EC and the perchlorination technique does not provide compositional information and can result in the detection of PCBs from the chlorination of artifacts. The recommended technique by GC/EC is the quantification of the PCB pattern as a commonly encountered PCB formulation, usually in North America, Aroclor 1254 or Aroclor 1260. The response is measured as the sum of several (i.e., three or more) prominent GC peaks and this is related to the response to a known quantity of internal standard.

GC/MS/SIM uses a similar approach with a series of characteristic ion chromatograms. The appearance in the appropriate florisil fraction of peaks within the determined retention time windows is taken as confirmation of the PCB and quantification is based on the PCB/internal standard response taken.

The quantification of individual PCB compounds is possible by high resolution GC/MS/SIM and this gives the greatest accuracy as no compositional approximations are necessary. However, such data is difficult to compare to existing data based upon whole PCB analyses and this approach, while attractive, has not been extensively used.

8. MEASUREMENT OF SEDIMENT GRAIN SIZE

8.1 Introduction

Grain size is an important and fundamental sediment property that has application in a number of areas. Grain size distributions offer information on despositional environments, sedimentation processes and on the geotechnical properties of the bulk sediment. In environmental studies, sediment grain size is important in comparing and evaluating chemical composition and benthic biota. Many environmental pollutants are scavanged from the water column through sorption processes with settling particulates. The effective surface area of particulates increases in inverse proportion to particle size so that the finer particulates have a much larger effective surface area for a given weight of material. The finer size particulates also include phases with a higher trace metal content than those of the coarser material. As a result, most trace metals and organics are partitioned into the fine size range (silt and clay) and are not evenly distributed within the sediment matrix. A comparison of the chemical composition of sediments and associated benthic communities therefore must be made on the basis of grain size characteristics.

It is usual to describe sediments on the basis of their gross grain size characteristics as gravel, sand, silt, clay or combinations thereof. These terms correspond to a broad range of particle sizes (generally taken as mean particle diameter) ranging from less than a micron to several centimetres. In North America, the Wentworth classification system is most widely used to describe grain size characteristics. The definition of sediment types in terms of particle diameter using the Wentworth classification is shown in Table 8.1. Actual methods for grain size analysis measure quite different properties of individual particles which relate in various ways to actual size. Sieving techniques are the most popular method and discriminate sediment particles on the basis of their smallest cross-sectional diameter. Settling tube or sedimentation methods measure the settling velocity of particles in water. Electronic particle counting methods determine particle size on a volume basis. Microscopic analysis of fines gives measurements which are most directly related to actual grain size as well as information on grain shape.

TABLE 8.1

RELATIONSHIP BETWEEN VARIOUS SIZE UNITS, U.S. STANDARD SIEVE SIZES AND WENTWORTH CLASSIFICATION

	U.S. STANDARD SIEVE MESH	MILLI-METERS	PHI (ϕ) UNITS	WENTWORTH SIZE CLASS	COMMON TEST PROCEDURES
GRAVEL	5	16	-4	Pebble	Sieve analysis
		4	-2		
	6	3.36	-1.75		
	7	2.83	-1.50		
	8	2.38	-1.25	Granule	
	10	2.00	-1.0		
SAND	12	1.68	-0.75		
	14	1.41	-0.5	Very Coarse Sand	
	16	1.19	-0.25		
	18	1.00	0.0		
	20	0.84	0.25		
	25	0.71	0.5	Coarse Sand	Settling Tube or Sieve
	30	0.59	0.75		
	35	0.50	1.0		
	40	0.42	1.25		
	45	0.35	1.5	Medium Sand	
	50	0.30	1.75		
	60	0.25	2.0		
	70	0.210	2.25		
	80	0.177	2.5	Fine Sand	
	100	0.149	2.75		
	120	0.125	3.0		
	140	0.105	3.25		
	170	0.088	3.5	Very Fine Sand	
	200	0.074	3.75		
	230	0.0625	4.0		
SILT	270	0.053	4.25	Very Coarse Silt	Sedimentation techniques: Pipette, Hydrometer; Optical Sensors X-ray
	325	0.044	4.5		
	-	0.037	4.75	Coarse Silt	
	-	0.031	5.0		
	-	0.0156	6.0	Medium Silt	
	-	0.0078	7.0	Fine Silt	
	-	0.0039	8.0	Very Fine Silt	
CLAY		0.0020	9.0		
	-	0.00098	10.0	Clay	
	-	0.00049	11.0		Practical Limit of Sedimentation Analysis
	-	0.00024	12.0		
	-	0.00012	13.0		
	-	0.00006	14.0		

$\phi = -\log_2$ diameter (millimeters)

No one method is capable of measuring grain size over the full range of gravel to clay size particles. For a detailed analysis of sediments with broad range of particle size, at least two methods will be required. Traditionally, sieving methods have been used to separate particles in the sand and gravel size categories (down to about 63 µm) while pipet and hydrometer sedimentation techniques have been used for size measurements of the clay and silt fractions (0.5 µm to 63 µm). Sedimentation techniques can also be used for sand analysis (particles to 2 mm) and numerous automated methods have been developed using this principle.

Electronic particle counting techniques have a wide theoretical operating range. These methods have become fairly standardized because of the available instrumentation and are a recommended method of the EPA (Plumb, 1981). Sieve, sedimentation and electronic counting techniques are the methods considered in the following. Microscopic procedures have not been included because of their limited use and expensive instrumentation requirements. Further information on these techniques can be found in Swift et. al, (1972).

8.2 Selection of a Suitable Method

The main factors to be considered in selecting a method for grain size analyses are:

- **detail required and type of sediment**
- **number of samples to be analyzed**
- **amount of material available**
- **availability of previous data and type of method used**
- **cost**

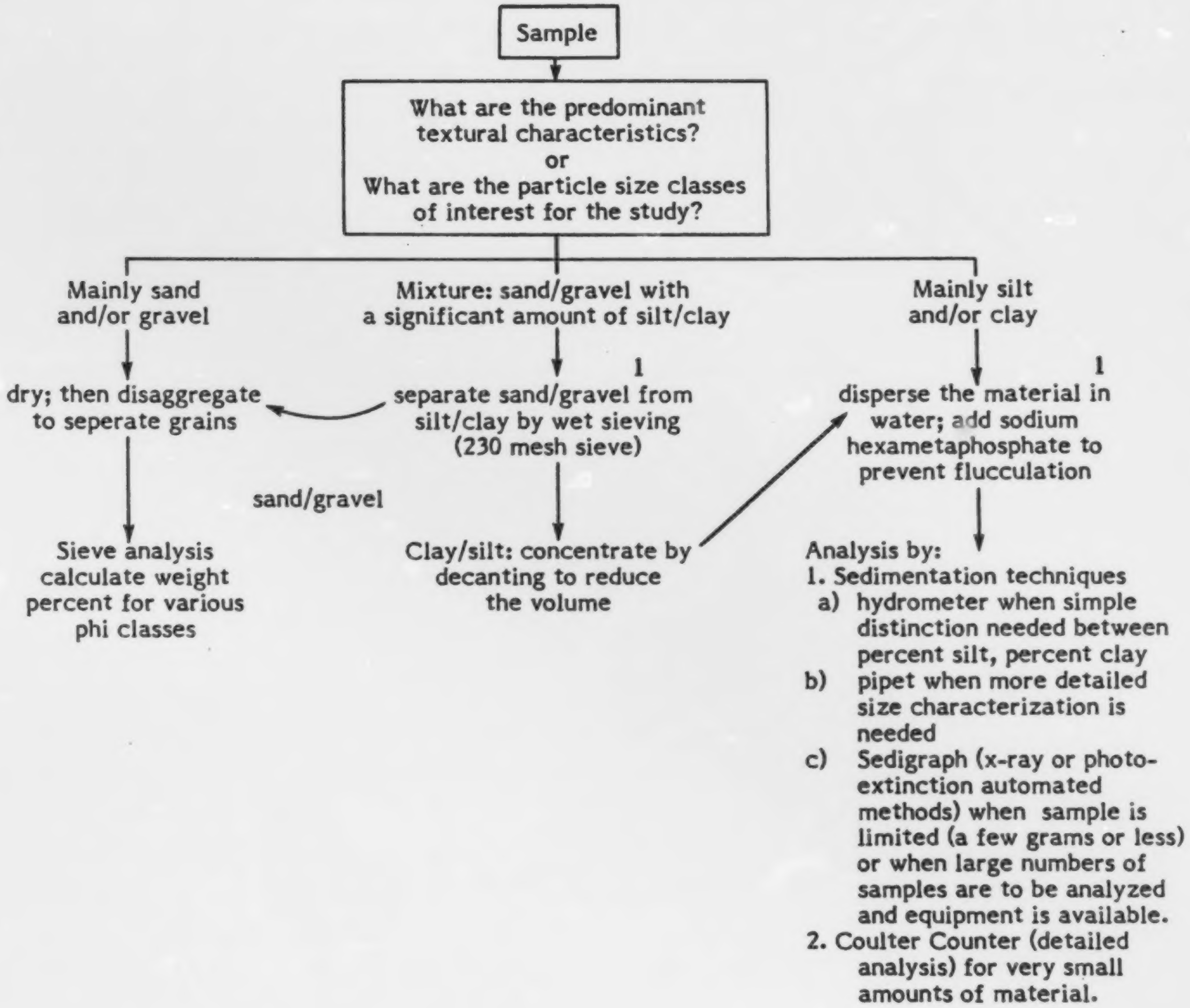
Grain size analyses in environmental sampling are usually carried out as supporting information. Samplers are chosen to meet the requirements of other parameters (benthos, trace metals or hydrocarbons), not grain size. The method of grain size analysis must usually fit the available sample size. Number of samples and cost are related. If a very large number of samples are to be analyzed, then automated methods have definite advantages in terms of time per analysis and hence cost. If analyses are to be done in a commercial laboratory, usually only classical sieve or pipet analyses can be done. Sieve and pipet or hydrometer analysis will also be the methods used in almost all historical data. Figure 8.1 provides a guide to method selection on the basis of particle size and size of sample. Advantages and disadvantages of the various methods and background information is given in Volume 1, Section III-4. A summary of performance characteristics, sample requirements and relative cost for the most common methods (sieve, pipet and hydrometer) is given in Table 8.2.

8.3 Sample preparation for Grain Size Analysis

Samples for grain size analysis are generally stored cool or frozen in a sealed container to prevent moisture loss. Sample size will vary with the type of sediment and analysis technique. Course sediments will require from 50 - 100 g or more for sieve analysis, while 20 g or less will suffice for fines by pipet analysis.

Sample preparation methods are somewhat arbitrary and depend on the final use of the data, properties of the sediment and the precision required. A sedimentologist, for instance, would wish to break-up any particulate aggregates while the environmentalist may wish to know environmental particle size and therefore aggregate size. In all cases however, the goal of sample preparation is to ensure that the measurements made of size distribution reflect natural variations and are not artefacts of sample preparation (Nelsen, 1983). Nelsen emphasized that a consistent manner of sample preparation is crucial to the final interpretation of data. Depending on the sediment type and the degree of splitting or dilution required, sample preparation times may equal or greatly exceed analysis time. This is especially true in automated methods.

Walton (1978) outlined a generalized scheme that is widely used for the preparation of samples for analysis by sieve and pipette techniques (Section III - 4, Volume 1). Because of its widespread use, this method is recommended. Samples are usually wet-sieved initially through a coarse 16 mm mesh sieve to remove cobbles. The sediment passing the sieve can be treated in a number of ways before further analysis and depending on the techniques to be used. The goal of these procedures is to prevent particles from aggregating or sticking to equipment. Organic material if present in large quantities (1-2% or more) is usually removed, generally by boiling with H_2O_2 (exact procedures can be found in Walton, 1978 or Carver, 1971). Depending on the method to be used, the remaining sediment is then wet sieved to separate the "fines" from gravel and sand for sieve and pipet analysis or just to separate gravel for settling tube or electronic particle counting methods. If analysis


Sample
What are the predominant textural characteristics? or What are the particle size classes of interest for the study?
Mainly sand and/or gravel
Mixture: sand/gravel with a significant amount of silt/clay
Mainly silt and/or clay
dry; then disaggregate to seperate grains
separate sand/gravel from silt/clay by wet sieving (230 mesh sieve) 1
disperse the material in water; add sodium hexametaphosphate to prevent flucculation 1
sand/gravel
Sieve analysis calculate weight percent for various phi classes
Clay/silt: concentrate by decanting to reduce the volume
Analysis by:
1. Sedimentation techniques
a) hydrometer when simple distinction needed between percent silt, percent clay
b) pipet when more detailed size characterization is needed
c) Sedigraph (x-ray or photo-extinction automated methods) when sample is limited (a few grams or less) or when large numbers of samples are to be analyzed and equipment is available.
2. Coulter Counter (detailed analysis) for very small amounts of material.

Note:

1) Preparation times for a mixed sediment or for techniques requiring a small amount of material will be much longer than the analysis time.

Figure 8.1 Choice of Grain Size Analysis Procedure

TABLE 8.2

SAMPLE REQUIREMENTS AND PERFORMANCE CHARACTERISTICS OF SIEVE AND PIPET AND HYDROMETER SEDIMENTATION TECHNIQUES

	PIPET	HYDROMETER	SIEVE
Sample Requirements	5 - 25 g	50 - 100 g	50 g sand 100 g gravel
Practical Range of Application	0.5 - 63 µm	~1 - 63 µm	~50-16,000
Time Required	almost 3 days required to carry out analyses to 0.5 µm; 17 hours to 1 µm.	slower than pipet analysis for equivalent particle size (24 hours to 0.1 µm)	~4 hours for detailed analysis
Comments	more precise than hydrometer because of less disturbance of water column. precision (CV) of approx. 0.5 ϕ attainable	less precise but less exacting procedure than pipet. Suitable for a field lab as no drying or weighing required	most economica and common method for sand analysis: only practical means of gravel analysis
Cost	$60-120/sample depending on detail	$40-60/sample	$35-50/sample
References	Walton, 1978; Krumbein and Pettijohn, 1938; Carver, 1971 Folk, 1974	Head, 1981; Krumbein and Pettijohn, 1938	Walton, 1978

of silt and clay size particles is required, the fines passing a 230 mesh sieve must be concentrated, either by centrifuging or allowing time for settling and then decanting excess water. Either method is time consuming especially where a large volume of water has been used in wet sieving.

For sieving, or settling tube methods the sand and gravel fraction is usually dried, weighed then disaggregated before analysis. Disaggregation is usually not a problem with clean sand and gravel.

The fine fraction if dried in an oven tends to be cohesive especially if there is a lot of clay present and the method of disaggregation critical to the final result. Freeze drying is preferable to oven drying for samples with a high clay content. Low temperature ashing is also an effective means of sample preparation (Kranck and Milligan, 1979). Nelsen (1983) compared four methods for disaggregation namely 1) soaking with Calgon, 2) shaking in water with a small amount of Calgon 3) ultrasonification and 4) stirring with a malt-mixer type stirring device. On the basis of the tests, Nelsen reached the following conclusions:

1. Under similar conditions of disaggregation and analysis there is no statistically significant difference between the size distributions of freeze or air-dried portions of a given sediment.
2. The disaggregation time needed to arrive at the terminal distributions seem to partition the methods into two groups, the very rapid (stir and ultrasonify) and the variable (shake and soak) disaggregation times.
3. From the data available the methods with variable disaggregation times seem to be sample dependent.
4. For a given sample the terminal distributions produced by all the disaggregation methods were statistically indistinguishable at the 95% confidence level.

The addition of a dispersant is usually required. The most commonly used dispersant is sodium hexametaphospate (Calgon) which is added to a suspension of the fines in sufficient quantity to inhibit aggregation and accelerated settling velocities.

Techniques using very small samples such as electronic counting and automated settling tube methods require more care in sample preparation as the population size is much smaller. This will involve microsplitting of samples to obtain representative subsamples which is more time consuming than subsampling for much larger weights.

8.4 Data Presentation

There are numerous means of presenting grain size data depending on the intended use and detail required. **As a minimum for environmental studies, the following should be provided:**

(1) tables of actual weights used and the weights in various size fractions.

(2) for settling tube methods, data on timing and methods of calculation (assumptions used).

Graphical presentations of data should either be in the form of a cumulative frequency distribution curve or a triangular plot (see Volume 1, Section III - 4) as these have widespread use and are useful for comparing a large number of samples at one time.

9. LEAD-210 AS A MEASURE OF SEDIMENT ACCUMULATION RATES

9.1 Background

The rate at which sediment accumulates is an important consideration in evaluating depth related features in sediment cores and in predicting the impact of anthropogenic inputs relative to natural fluxes. There are a number of methods for estimating rates of sediment accumulation depending on location and sediment characteristics. Direct measurements of the downward particle flux can be made by trapping settling water column particulates. Estimates can also be derived from stratigraphic inferences based on total depth of sediment in relation to the estimated time of deposition or from budget calculations relating to the particulate flux associated with river run-off and the concentration of particulates in the water column. Finally analysis of sediment cores can be made based on paleontology and radiometric methods. In general, no single method is universally applicable and it is desireable to obtain estimates from two or more methods to improve reliability. The determination of geochronologies using artificial and natural radionuclides has become well established as a means for dating marine deposits over periods of time of tens to millions of years (Turekian and Cochran, 1978; Goldberg and Bruland, 1974). From an environmental perspective, recent processes are most important and this reduces the number of useful radionuclides considerably.

Lead-210 is the most commonly used natural radionuclide for dating recent sediments. Its chemistry and half-life permits dating for periods of 100-150 years. Pb-210 methods have been reviewed in detail by Robbins (1978) and have been applied to a variety of lake and coastal marine deposits. Dating using a natural radionuclide is based on the decay of the isotope after incorporation into the sediment.

Lead-210 is a member of the U-238 natural radioactive series and has a half-life of 22.3 years. There does not appear to be a significant amount of Pb-210 produced in nuclear explosions.

Atmospheric fluxes of Pb-210 in arctic regions are lower than in more temperate regions. Naidu et al., (1981) have reported an average flux of 0.08 Bq cm $^{-2}$ y^{-1} for the Alaskan Beaufort Shelf. This is an order of magnitude lower than fluxes measured in the northeastern United States and California.

For practical purposes, the method provides estimates of time scales of up to 5 half-lives (∼ 100-110 years). In ideal conditions, (i.e. undisturbed sediments) the unsupported or excess Pb-210 concentrations exhibit an exponential decay in activity with depth of burial. However, sediments are often disturbed by biological or physical processess and by variation in sedimentation rate which purturbs the expected exponential decay curve. This can lead to problems in data interpretation or erroneous results. **For this reason, it is important that a sedimentation rate estimate be obtained from an alternate method for comparative purposes.**

9.2 Sampling for Pb-210

Sampling for Pb-210 dating places added emphasis on collecting the sediment surface intact. The factors to be considered when sampling and subsampling are summarized in Table 9.1.

9.3 Choice of Analytical Method

There have been two basic approaches to Pb-210 measurements in sediments. Neither involves the direct measurement of Pb-210 since it is a low energy β emitter (Emax = 61 kev). The most common approach involves the extraction and purification of Pb from the sediment via an acid leach and precipitation and counting of the β decay of Bi-210 (Emax = 1.17 MeV), the daughter product of Pb-210 decay. The other approach used involves extracting and isolating Po and Pb from the sediment and counting the α decay of Po-210 (Emax = 5.3 MeV) which is assumed to be in secular equilibrium with Pb-210. Determination of either Pb-210 or Po-210 is a multi-step process. Briefly, the metal is extracted from the sediment by a hot acid leach, purified by extraction from the leachate and then collected as a precipitate. Po-210 is measured directly, while Bi-210 is allowed to equilibriate with Pb-210. Recommended methods are summarized in Table 9.2. Two choices are given: 1) collection and direct measurement of Po-210, and 2) collection of Pb-210 and subsequent measurement of Bi-210 after a suitable equilibration time. **It should be stressed that whatever method is used, a suitable carrier or tracers (see Table 9.2) be used to determine the yield.** As the use of the method lies in relating changes in Pb-210 concentrations, it is imperative that variations in yield be taken into account.

TABLE 9.1

FACTORS/RECOMMENDED PROCEDURES FOR Pb-210 SAMPLING, SUBSAMPLING AND STORAGE

STEP	RECOMMENDED PROCEDURE	COMMENTS/REFERENCES
Type of Sediment	only sediments with a high proportion of silt and clay should be considered for dating	Pb-210 levels in Arctic sediments are generally low. Pb-210 as for other trace elements, is concentrated in the silt/clay fraction
Length of Core Required	20 - 50 cm	length must be sufficient so that excess Pb-210 will have decayed away. This will take 100-150 years. Sedimentation rates will rarely exceed 1 mm/y.
Type of Sampler	1. Box corer or damped cylindrical gravity corer 2. cylindrical gravity corer with slowed entry.	Section 3 (Choice of Samplers) It is crucial that the core be undisturbed and that surface "flocs" and fines be recovered.
Handling of Core in Field	1. keep upright 2. carefully draw off surface water with a siphon	retain some surface water for later salinity analysis (necessary for porosity calculation)
	3. skim off surface floc if present and store separately	plastic bottle/bag; and freeze
Storage	4. extrude core and section or freeze the entire core in the core barrel or liner after surface floc is removed. Core sections stored in plastic bags and frozen.	
Extruding Cores	A. IN FIELD	
	if convenient, cores can be extruded with a tight fitting piston device	Kemp et al., 1971 Before sectioning, material next to the sampler wall should be removed as it may be contaminated
	B. IN LAB	
	Frozen cores can be thawed slightly and then extruded and cut using a band saw or thawed completely and handled as in the field.	
Sectioning	thin sections (0.5 - 1.0 cm) are required for the upper 10 cm. Thicker sections can be taken at greater depths.	take as many sections as possible. All sections need not be analyzed
	Extruded, frozen cores may be cut with a band saw using carbide tipped blade.	
	Thawed or unfrozen core sections should be cut with a knife.	
Sample Requirements	Each section should yield at least 1-5 g dry sediment.	

TABLE 9.2

RECOMMENDED ANALYTICAL METHODS FOR Pb-210 DATING

METHOD	REFERENCE	QUANTITY OF SEDIMENT REQUIRED	DIGESTION	SEPARATION AND PURIFICATION OF Pb	RECOVERY/PRECISION	COUNTING	COST
1. Pb-210 via Bi-210	Koide et al., 1972 Krishnaswamy et al., 1971; Rama et al., 1961	2 - 10 g	Initial heating at 400°C for 2-3 hours to remove organics. Residue leached in 6N HCl for 1 hour at 100°C. Leachate dried and dissolved in 1.5N HCl	30 mg Pb^{2+} (Pb-210 free $PbNO_3$ solution) added as carrier: solution passed through anion exchange column: Pb elected with 1.5N HCl: Pb is precipitated at Pb 504 from pH 2 solution	not stated	made after allowing 20-40 days for in growth of Bi-210 daughter in flow-type beta counter.	\$50 - \$75/ sample
	Matsumoto and Wong, 1977. Macdonald et al., 1983	~3 g	20 mL conc. HNO_3 at 120°C for 2 hours: leachate diluted to 3N HNO_3	20 mg Pb^{2+} (Pb-210 free $PbNO_3$ solution) added as carrier: Pb separated by electrodeposition on a Pt anode as PbO_2. PbO_2 dissolved in 3N HNO_3 containing a few drops of H_2O_2 Pb precipitated as Pb S04 from pH 2 solution	70-95% recovery of Pb	made after allowing 30 days for in growth of Bi-210 daughter in flow-type beta counter	~\$100/sample
2. Pb-210 via Po-210	Robbins and Edgington, 1975 Cliffton and Hamilton, 1979 Flynn, 1968	up to 5 g	HNO_3 followed by several aliquots of conc. HCl. HCl treatment continued until no NO_2 fumes evolved. Solution diluted.	- measurement of Po-210 - assumed to be in secular equilibrium with Pb-210. Filtrate is evaporated to small volume and heated with conc. HCl to eliminate any remaining HNO_3. Po-210 self plated onto silver planchettes with 10 mg of a bismuth carrier at 85-90°C in the presence of sodium citrate at pH 2. Plating times 60 min for 79% recovery.	quantitative recovery of Po-210	Po-210 alpha-activity counting done for 10^3 min. (2)	\$75-\$100/sample
	Nriagu et al., 1979	1 - 3 g	15 mL aqua regia/g sediment heated at 90°C for 6 hours with stirring	plating times 4 hours with shaking in presence of ascorbic acid (1 g/3 g sediment)		NMC 2 proportional counter	
	Farmer, 1978	1 g	100 mL 8M HNO_3 for 4 hours at 70°C	Pb-208 added during digestion to monitor yield			

9.4 Data Interpretation

There have been three basic models developed for interpreting Pb-210 depth profiles and estimating accumulation rates (Appleby and Oldfield, 1983); 1) constant flux, constant sedimentation rate (CF:CS) model; 2) constant rate of supply (CRS) model and 3) constant initial concentration (CIC) model. The models and their application and limitations are described in Volume 1 and references therein.

No single model is applicable in all cases, while in some instances no model may be suitable. Appleby and Oldfield (1983) have outlined a tentative procedure for choosing which model to apply:

1. Linear profiles (In excess Pb-210 activity vs depth): all models give the same chronology
2. Non-linear profiles
   a) if the total cumulative excess Pb-210 (residuals) in a core is comparable with known atmospheric flux or with levels in nearby cores, then the C.R.S. model is likely applicable.
   b) if Pb-210 residuals vary from different sites and are in proportion to sediment accumulation rates, then the CIC model is most likely applicable.
   c) if neither case (a or b) is satisfied, dating cannot be reliably carried out.

Although the CRS and CIC models make one less assumption than the CF:CS model, they require more detailed sampling intervals and are subject to false interpretation. Physical and infaunal mixing as well as episodic events such as slumping or ice scouring would result in non-linear (ln A vs z') plots and could be interpreted as changes in sedimentation rate or flux. For these reasons, the dating of sediment cores must be done with caution and supporting data sought from independent dating methods and other measurements which might indicate whether or not the sediment has been mixed or disturbed. Swan et al, (1982) have noted that supported Pb-210 may not be constant with depth and that Ra-226 measurements should be made to compensate for this.

10. QUALITY ASSURANCE IN ANALYTICAL METHODS

Quality assurance is a term used to describe a program designed to reduce measurement errors and to assure that the results have a high probability of being of acceptable quality. The goal of a quality assurance program is to bring a measurement system into a state of statistical control and subsequently maintain it there. Statistical control implies that procedural errors have been reduced to acceptable levels and have been characterized statistically. A quality assurance program embodies two concepts: **quality control**, the mechanism established to control errors; and **quality assessment**, the system used to verify that control is working (Taylor, 1981). The topic of quality assurance has been discussed in detail in several recent reviews (Taylor, 1981; Keith et al., 1983; Kirchmer, 1983)

Quality has a subjective definition. Acceptable data in one study may be found lacking in another. There are no set rules that one can apply. It is only possible to outline some of the elements that go into quality control and assessment. The actual methods used will be dependent on the intended use of the data, the laboratory and demands of the situation. **However, some type of quality assurance program must be incorporated into all studies so that quality statements can be made.** If no information is available on the data which will allow statements of precision and accuracy, then the data is of little value.

A guide has been recently produced for obtaining, calculating and reporting quality statements for chemical analysis of marine sediments.[1] The guide includes discussions of general concepts, terminology, systematic and random errors, and provides detailed recommendations for quality control and assessment. Several worked examples are provided to illustrate the appplication to actual data sets. **This guide should be consulted in designing a quality assurance program.**

An important first step in the use of any analytical procedure is validation of the method. This means establishing key performance characteristics (accuracy, precision, detection limit, blank) and bringing the method into a state of statistical control (Taylor, 1983). Some specific quality control and assessment procedures that should be used in ongoing marine sediment and biota analyses are:

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[1] Macdonald, R.W. Guidelines for Obtaining, Calculating and Reporting Quality Statements for Chemical Analysis of Marine Sediments. A copy of this report may be obtained by contacting Dr. R. Macdonald, Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney B.C. (604)-656-8409.

a) analysis of reference sediments and tissue,

b) analyses of blind replicates and control samples,

c) use of control charts for assessing the quality of the results obtained for items a and b, as well as blanks and standard solutions and

d) collaborative testing programs.

For many organic compounds, reference materials are not available and quality assurance programs must emphasize components of the measurement system such as blanks, replicate analyses, spike recoveries and collaborative testing. It has been suggested that the cost of quality assurance should be 10-20% of the total analytical costs:

10.1 Certified Reference Materials

10.1.1 Metals

A list of reference materials (sediments and tissue) that have certified levels for key metals is given in Table 10.1. A list of suppliers is given in Table 10.3.

10.1.2 Organics

A list of reference materials and intercalibration materials (i.e., no certified values available) is given in Table 10.2 for organics. No certified reference materials are available at present for biota although some are under deveopment (Russell, 1984). Other reference materials that may be useful for instrumental calibration are also listed.

10.2 Documentation and Reporting

The requirements for documentation and data reporting are outlined in Macdonald's Guide. The key requirements are summarized below:

Table 10.1

Standard Reference Maerials for Metals in Marine Analytical Chemistry
(from Russell, 1984)

	Name	Metals (certified values are available)	Supplier
Sediments	Estuarine Sediment, SRM 1046	As, Cd, Co, Cu, Pb, Mn, Mg, Ni, V, Zn, Al, Ca, Fe, Mg, and P	NBS
	Marine Sediments, MESS-1	Be, V, Cr, Mn, Co, Ni, Zn, As, Sb, Cd, Hg, Pb, C, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti and Fe	NRCC
	Marine Sediments, BCSS-1	Be, V, Cr, Mn, Co, Ni, Zn, As, Sb, Cd, Hg, Pb, C, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti and Fe	NRCC
	Marine Mud, MAG-1	Al_2O_3, Fe_2O_3, B*, Ba, Be*, Cd*, Co, Cr, Cs*, Cu, Ga*, Hf*, La, Li*, Mn, Mo*, Nb*, Ni, Pb*, Rb, Sb*, Sc, Sn*, Sr, Ta*, Th*, U*, V, Y, Zn, Zr	USGS
Biological Materials	Oyster Tissue, SRM 1566	As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Ni, Rb, Se, Ag, Sr, U, Zn, Ca, Mg, K, Na	NBS
	Lobster Hepatopancreas, TORT-1	V, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Hg, pb, Na, Mg, P, S, Cl, K and Ca	NRCC
	Chlorella, NIES No. 3	K, Ca, Mg, Fe, Mn, Sr, Zn, Cu, Co	NIES
	Mussel Powder, NIES No. 6*	Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr, Ti, Zn, Ca, K, Mg, Na, P	NIES
	Albacore Tuna, RM 50*	Se, Zn, As, Hg, Mn, Na, K, Pb	NBS

* Research material, values not certified.

Table 10.2

Available Certified Reference Materials and Intercalibration Samples for Organics

a) Certified Reference Materials

a.1 Sediments

I.D.	Source Agency	Certified Components	Comments
CS-1	NRC Canada	PCB's (Aroclor 1254)	Coastal marine sediment dried, homogenised
HS-1	NRC Canada	PCB's (Aroclor 1254) PCB's 10 individual isomers	Harbour sediments
HS-2	NRC Canada	PCB's, Aroclor 1254 PCB as 10 individual isomers	Harbour sediment
EC-1	CCIW	Organochlorine compounds PAH, PCBs chlorobenzene	In preparation Lake sediment
EC-2	CCIW	Organochlorine compounds PAH, PCBs chlorobenzenes	In preparation Lake sediments

a.2 Biota

None available

b) Intercalibration Reference Materials

b.1 Sediments

I.D.	Source Agency	Certified Components	Comments
Duramish II	NOAA/BLM	alkanes PAH isoprenoids	River sediment

b.2 Biota

I.D.	Source Agency	Certified Components	Comments
MA-A-1	IAEA	5 chlorinated pesticides 2 PCB's (Aroclor 1254)	Cocepod Homogenate 15 labs participated
MA-M-2	IAEA	7 chlorinated pesticides 2 PCBs (Aroclor 1254,1260)	Fish flesh homogenate 15 labs participated
Capelin Oil	ICES	7 chlorinated pesticides 1 PCB (aroclor 1254) (21 compounds identified)	44 labs participated 3rd ICES intercalibration sample
Mussel	ERNL/USEPA	26 PAH, total aromatics PCB (Aroclor 1254) DDE, total saturates	Accepted values based on mean results of 4 lab.

c) Other Reference Materials

I.D.	Source Agency	Certified Components	Comments
SRM 1580 (Shale Oil)	US NBS	5 PAH 2 phenols 2 quinolines	Certified concentrations of organics in shale oil
SRM 1647 (Priority pollutant PAH)	US NBS	16 PAH	Certified concentrations of PAH in acetonitrile
SRM 1649 (Urban dust)	US NBS	5 PAH	Certified in urban dust

Table 10.3

Addresses of Reference Material Suppliers

Analytical Quality Control Services
International Atomic Energy Agency (IAEA)
Laboratory Seibersdorf
P.O. Box 590
A-1011 Vienna, Austria

International Council for the Exploration of the Sea (ICES)
Palaegade 2-4,
DK 1268
Copenhagen, Denmark

Marine Analytical Chemistry Standards Program
National Research Council of Canada (NRCC)
Division of Chemistry
Montreal Road
Ottawa, ON
K1A 0R9

National Bureau of Standards (NBS)
Office of Standard Reference Materials
Washington, DC 20234
USA

National Institute for Environmental Studies (NIES)
Japan Environment Agency
P.O. Yatabe Tsukuba
Ibaraki 300-21
Japan

National Water Research Institute
Canada Centre for Inland Waters (CCIW)
867 Lakeshore Road
P.O. Box 5050
Burlington, ON
L7R 4A6

NOAA/BLM
Northwest and Alaska Fisheries Centre
Environmental Conservation Division
2725 Montlake Boulevard East
Seattle, Washington
98112

ERNL/USEPA
Quality Assurance Branch
U.S. Environmental Protection Agency
Cincinnati, Ohio
45268

US Geological Survey (USGS)
National Center
Stop 972, Reston, VA 22092
USA

a) **Documentation of analytical measurements should provide information sufficient to support all claims made for all the results.** Documentation requires all information necessary to (a) trace the sample from the field to the final results, b) describe the methodology used, c) describe the confirmatory evidence, d) support statements about detectability, e) describe the Quality Assurance program and demonstate adherence to it, and f) support confidence statements for the data. Methods of determining detection limits, accuracy and precision must be clearly stated and outlined.

b) **Laboratory records should be retained in a permanent file for a length of time set by government, other legal requirements, or the employing institution, whichever is longer.**

c) **Measurement results should be expressed so that their meaning is not distorted by the reporting process.** Reports should make clear which results, if any, have been corrected for blank and recovery mesurements and calibration conversions. Any other limitations should also be noted. **As a minimum, the formula used should be given.**

d) **Data should be reported only to the number of significant figures consistent with their limits of uncertainity.** State the uncertainty to two significant figures and the reported value to the last place in the uncertainty statement.

e) **When appropriate, the relationship between individual sample values, blanks, recoveries, and other supporting data should be shown.** If possible, and within the scope of desired resuls, a number of mesurements sufficient for statistical treatment should be made. When this is not the case, an explanation is necessary including complete details of the treatment of the data. If average values are reported, an expression of the precision, including the number of measurements, must be included. **The minimum requirements for data to be used for statistical comparisions is the mean, standard deviation and number of replicates.**

f) **Data should be reported using S.I. units, where appropriate.**

g) **If a published methodology is used, it should be cited.** Any modification, as well as any new methodology or new approach to the making of measurements or interpreting the results, must be described in detail, including test results and details of its validation. Instrumentation should be described.

The cost of documenting and reporting will vary with the interpretive detail required. An allowance of 10-20% of the total budget should be made for reporting.

11. SELECTION, MAINTENANCE AND COLLECTION OF ORGANISMS FOR BIOASSAYS

11.1 Selection of Species

The review of the literature (Volume 1, Section 7) revealed that no single species is suitable for all bioassay tests. Nevertheless certain criteria can be established to help select appropriate species. These criteria are summarised in Figure 11.1 and outlined below:

The investigator should develop a set of criteria to be met and then select the most appropriate organism. As it is unlikely that any one species will meet all of the requirements, it is important to consider using two or more species. Some important questions to answer in selecting suitable organisms are:

(1) What is the reason for the bioassay? If the bioassay is for regulatory compliance then fish or a required organism must be used.

(2) In what environment is the material expected to have the most serious impact? An ecologically important or economically important species living in that environment (e.g., pelagic - fish; benthic -crustacea), should be chosen.

(3) What effect is to be examined? A species in which this effect is most easily observed or interpreted (short-term lethal effect, long term effect, bioaccumulation, sublethal short effect - fish, crustacea, bivalves, fish respectively), should be chosen.

(4) Is the test species suitably sensitive to the substance under study?

(5) How well documented is the life history of the selected test species? Pick a species that has a well documented life history over one that does not.

(6) How easily maintained is the species in the laboratory?

(7) How widely is the species distributed within the environment? Meeting this criterion allows differing laboratories to use the same test species.

A major criterion is the end use or goal: if the biological assay is to be conducted for regulatory compliance **then the choice will be dictated by the regulatory agency.** It has been strongly recommended that the Threespine Stickleback (Gasterosteous aculeatus) be used as the test organism in the Arctic, since it is small (5 cm long) and hence convenient for laboratory use, lives in salt water and is found on the arctic coast of North America (Wong, 1982). Although this species is recommended as a standard assay organism alternate species could be used.

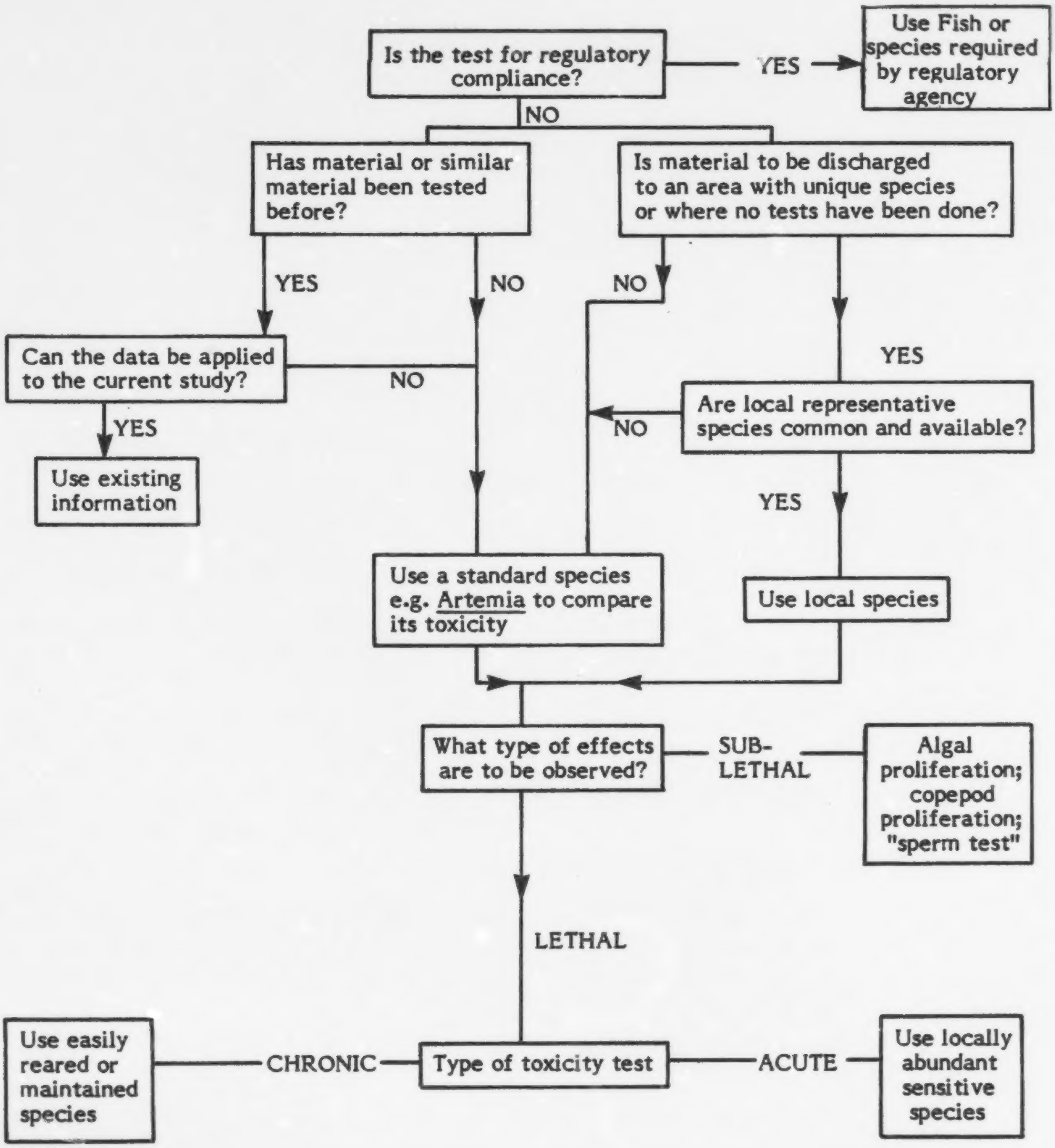
Is the test for regulatory compliance?
YES
Use Fish or species required by regulatory agency
NO
Has material or similar material been tested before?
Is material to be discharged to an area with unique species or where no tests have been done?
YES
NO
NO
Can the data be applied to the current study?
NO
YES
YES
Use existing information
Are local representative species common and available?
NO
YES
Use a standard species e.g. Artemia to compare its toxicity
Use local species
What type of effects are to be observed?
SUB-LETHAL
Algal proliferation; copepod proliferation; "sperm test"
LETHAL
Use easily reared or maintained species
CHRONIC
Type of toxicity test
ACUTE
Use locally abundant sensitive species

Figure 11.1. Flowchart for selecting test organisms for bioassays.

The sensitivity of the test organism is important. If only fish are used then under-estimations of the toxicity of substances could be obtained as fish are in general less sensitive than many other organisms to contaminant inputs.

Using either locally available ecologically important or economically important species as test organism is also a consideration as materials may be discharged into a new environment having a unique assemblage of species on which no bioassay tests have been conducted. In order to have an idea of the significance of the resulting data, they could be compared against results of a similar bioassay in which fish are used. In addition, reference toxicants can be used to provide a standard, facilitating comparisons and intercomparability among different laboratories.

The expected site of impact is also an important factor. A reason for using other species in addition to the stickleback or other fish which live in the pelagic environment is that the water column may not be as seriously impacted as the benthic environment. Hence it is important to consider using a benthic-dwelling organism as the test species when assays are being conducted on solid material that will rapidly settle out.

Finally, the type of effect that is to be investigated plays an important role in species selection. If short-term effects are to be studied then tests of lethality can be conducted (e.g., acute toxicity tests). In this instance, it is important to use a species that has easily recognisable indications of death or approaching death. Both fish and crustacea meet this criterion in that either opercula movement in the fish ceases or the fish turns on its back or both while in crustacea it is often easy to observe in ceasation in the movement of the animal or its appendages. The same can not be said of molluscs or enchinoderms. However, if long term effects are to be studied then molluscs may be better suited. For example, bivalves are well suited for studying the bioaccumulation of pollutants. Observations of the long-term effects over the lifetime of an organism are most easily conducted using relatively short-lived species such as copepods. Algae are also suitable for this type of work. If short-term, sublethal physiological effects are to be examined (e.g., enzyme inhibition or induction) fish are probably the most suitable test organism as physiological responses to pollutants are well documented in these organisms as compared to the invertebrates.

11.2 Collection and Maintenance of Organisms for Bioassays

11.2.1 General Considerations

a) Collection and Handling

Since intact, healthy organisms are of prime importance for successful laboratory maintenance and subsequent bioassays, trauma during collection and any consequent handling must be reduced to a minimum. A detailed discussion of methods for sampling the benthic environment is given in Sections II-3 and II-4 of volume 1 as well as in Holme (1964).

Commonly, a variety of biological dredges and trawls are used for exploratory qualitative sampling of the epifauna. These devices skim the surface of the seabed and are effective for collecting larger or scarcer epifauna and fast-moving animals such as bottom living fish, cephalopods and crustaceans. Dredging and trawling in arctic waters is, of course, limited to open water conditions. A detailed explanation of the different types of gear as well as techniques and methods for their use is provided in Volume 1, Section II-3.2.1.

A grab sampler is the conventional and best means for qualitatively sampling benthos on soft bottoms. A description of the gear, advantages and disadvantages of the various grabs and factors to consider in grab selection are given in Volume 1, Section II-3.3.1 and in sections 3 and 5 of this Guide. Factors associated with arctic sampling tend to favour the van Veen grab for general use.

Diving is probably the best technique for investigating the macrobenthos of hard or gravelly substrates which cannot be adequately sampled by dredge or other means (see Volume 1, Section II-4 for further details). Hand collection by divers is a very effective means of obtaining specimens in good condition for laboratory studies. Suction devices combining an airlift and a pump can be used by divers for the careful collection of non-attached forms and for sampling the infauna in soft bottoms. This method of collection can yield animals which are not visibly injured and which appear to be in good physiological condition (see Schneider and Hanes, 1981). Divers can also assist in the collection of fish.

The collection of pelagic organisms, including phytoplankton, zooplankton and fish is best accomplished by the use of various types of nets.

Baited traps are often ideal for collecting the smaller, faster-moving members of the epibenthic fauna such as amphipods, isopods and decapod crustaceans.

Sediment samples collected with grabs or dredges must be processed to remove the organisms. Extraction of the infauna usually involves emptying the contents of the dredge or grab onto a sieving table whereby a gentle stream of water from a sprinkler system or hose removes the surrounding sediment, leaving the specimens intact. Washing the sample through a nest of screens of various mesh size will aid recovery of smaller organisms.

b) Maintenance

The successful laboratory maintenance of organisms largely depends on how well natural environmental conditions can be simulated. The four essentials of successful maintenance are:

1) the water (medium) used,
2) the management of its life-supporting qualities,
3) adequate nutrition of the organisms, and
4) proper equipment.

Any changes in water quality must be counteracted by mechanical, biological or physico-chemical treatment. Kinne (1976) gives a detailed description of water quality management and technology in relation to the cultivation of marine organisms.

There are two basic categories of aquatic culture systems: still-water and running-water (open or closed). Only bacteria, unicellular plants and a variety of small invertebrates can be maintained in still water. Flow through (open) systems are advantageous but are not absolutely necessary as long as the water is filtered and water quality is maintained. Recirculating (closed) systems (see King, 1975 and Kinne, 1976) offer easier control and monitoring of many environmental parameters and they can be established far from a salt water source.

In the laboratory the animals should be allowed to acclimate before they are used as test organisms. During maintenance, overcrowding of the test organisms must be avoided. Careful attention should be paid to maintaining a suitable water temperature, particularly in arctic species which may have a very narrow range of temperature tolerance.

Adequate nutrition and disease control also constitute major problems in the laboratory maintenance of organisms. Phytoplankton stocks can be used to maintain certain filter feeders such as pelecypod species. Guillard (1975) describes relatively simple and reliable methods for the culture of marine phytolankton species for feeding marine invertebrates. Mixtures of phytoplankton and larger zooplankton can provide an adequate food supply for other species of bivalves and barnacles. A beef heart extract (McCammon, 1975) has been used with great success as a supplemental food for filter feeders which cannot subsist long on plankton alone and for other invertebrates that prefer a more solid diet.

Disease causing microorganisms can be inhibited or eliminated by the use of antibiotics. Streptomycin and penicillin are used most frequently, streptomycin being better suited for the crude culture of marine invertebrates because it is a wide spectrum antibiotic with a relatively long half-life and is not readily deactivated at alkaline pHs. D'Agostino (1975) discusses the application of various types of antibiotics in the culture of invertebrates. It is usually a good idea to include a fungicide in antibiotic mixtures to control fungal growth.

A more detailed discussion of the collection and maintenance of various species to be used as test organisms in bioassays can be found in Volume 1, Sections III-7.3 and 7.4.

11.2.2 Recommended Methods

a) **Microalgae**

a.1 Collection and Handling

Sampling the phytobenthos, in particular, benthic marine microalgae, can be accomplished by collecting sediment samples with a corer and then separating the different types (see Volume 1, Section II - 5.2.2). Usually the collection of the pelagic forms of the microalgae in the phytoplankton is carried out by using nets. The microalgae are then carefully separated from the plankton tow samples. Also, pelagic microalgae can be obtained from plant fragments or other debris in the water or by sampling naturally ocurring blooms.

After collecting the crude sample, the next step is to obtain uni-algal clone cultures and then bacteria-free cultures. Ukeles (1976) defines a variety of methods for isolating representatives of single algal species and for the purficiation of algal species from contaminating organisms as well as media preparation, sterilization and subculturing.

a.2 Maintenance

Conventional culture methods for marine unicellular algae involve the inoculation of solid or liquid media dispensed into various types of culture containers, including test tubes, flasks or carboys.

Antibiotics alone or combined with detergents are often used to purify contaminated cultures, as a direct treatment or in conjunction with some other purification technique.

The nuitritional requirements for microalgae include a varying number of mineral elements (probably 15 to 20), an inorganic nitrogen source, trace metals, a carbon source, vitamins and Na, K, Mg and Ca (see Ukeles, 1976 for more details).

Ukeles (1976) discusses the physical aspects - illumination, temperature, salintiy and pH - which are necessary for the culture of microalgae.

a.3 Suggested Arctic Species

Phaeodactylum tricornutum, Skeletonema costatum and Dunaliella tertiolecta are three of the most common microalgal species which are used as assay organisms. If these are not available from arctic waters then closely related species should prove to be acceptable bioassay organisms. As part of the Baffin Island Oil Spill (BIOS) Project in situ studies were conducted to assess the effects of oil and dispersed oil on primary productivity of the ice algae community (Cross, 1982; Cross and Martin, 1983). The ice algal community was dominated by pennate diatoms, in particular, Nitzschia grunouri and N. frigida.

b) Ciliated Protozoa

b.1 Collection and Handling

Protozoan organisms, e.g. ciliates, are commonly collected in sediment samples taken by corers or grab.

b.2 Maintenance

Kinne (1977) has tabulated the approximately twenty species of marine ciliates which have been cultured, the media they were grown in and the food they were given. Ciliates will adapt to various artificial seawaters when adjusted to the original salinity. Ciliate cultures can be maintained in clean and sterile Petri dishes, test tubes or various flasks. Provasoli (1977) discusses purification of ciliate cultures with various antibiotics, as well as various media which may be used for isolation and growth. Most ciliates feed phagotrophically (ingestion and subsequent digestion of solid particles of food) on bacteria but in suitable artificial media they can grow osmotrophically (absorption of organic substances in solution) on dissolved nutrients.

b.3 Suggested Arctic Species

Cristigera spp., common bacterivorous, sediment-living marine ciliates have been used as toxicity indiators for heavy metals (Gray and Ventilla, 1973; Gray, 1974). This ciliate, or a closely related species, should be an acceptable bioassay organism.

c) **Nematodes**

c.1 Collection and Handling

These worms are usually collected by dredge or grab. They can be removed from the sediment in the field using a gentle stream of water and sieving screens or can be carried to the laboratory in the sediment which acts as a temperature buffer. Either way, avoid temperature extremes by transporting them in insulated coolers.

c.2 Maintenance

Kinne (1977) has tabulated culture media and food sources for those marine and brackish nematodes which have been cultured. These worms have been grown on fungal mats in liquid culture as well as in a variety of agar media.

In the natural environment most nematodes feed on decaying or living microorganisms (bacteria, fungi) or microalgae associated with sediment habitats. Many free-living nematodes are carnivorous and feed on small metazoan animals, including other nematodes. In laboratory cultures bacteria, yeasts, flagellates, fungi, ciliates and diatoms have been used as food sources for nematodes.

c.3 Suggested Arctic Species

The potential role of marine nematodes as indicators of water-quality and pollution load has not been investigated to any great extent. Adequate culture methods remain to be worked out for most marine and brackish nematodes. However, Samoiloff et al. (1980) have described a long-term toxicity assay for aquatic contaminants using the nematode, Panagrellus redivivus and Tietjen and Lee (1984) have used two species of free-living nematodes as bioassay organisms for pollutant-contaminated estuarine sediments. Any abundant local arctic species, preferably related to the aforementioned worms, should be considered as possible test organisms for bioassays.

d) Polychaetes

d.1 Collection and Handling

Like the nematode worms, polychaetes are commonly collected by dredge or grab samplers. These worms can be separated from the sediment in the field or can be transported to the laboratory in the sediment which acts a a temperature buffer. A gentle stream of water (it should be the same temperature as the water from which the sample was collected) and sieving screens facilitate the removal of intact specimens. Avoid temperature extremes by transporting the worms in insulated coolers. The polychaetes, Pectinaria hyperborea and Brada villosa, have been collected for use in bioassays from near Barrow, Alaska using an airlift operated by a SCUBA diver (Schneider and Hanes, 1981). They were transferred immediately to buckets of freshly collected seawater to prevent thermal shock. The polychaete Melaenis loveni, has been obtained for use in toxicity tests by divers below the ice from the Prudhoe Bay area of the Beaufort Sea (Nortec, 1981).

d.2 Maintenance

It is preferable, although not always practical to place each specimen in its own container. Polychaetes are generally maintained in small enclosures which should be non-toxic and thoroughly cleaned; finger bowls and petri dishes are most commonly employed. Dean and Mazurkiewicz (1975) discuss in some detail the culture of polychaetes, both adults and larvae. An open sea water system, usually with a filtering system, is most commonly used for maintaining large numbers of adult polychaetes. Closed systems can also be used for maintenance and range from standing water kept in motion via aerators, stirrers or shakers to recirculating water systems with filters. The water should be changed frequently, especially if food is added. Adequate temperature control is a must for the successful maintenance of polychaetes.

Some form of substratum often enhances maintenance; a layer of detritus and/or fine sediment works well. Several artificial substrates, absorbent cotton and the mineral, cryolite, have been used successfully. Artificial burrows of plastic or glass tubing can be used to maintain nereids, arenicolids, some spionids and glycerids.

Many species of worms can be kept for short periods without food; extended periods without the addition of food results in a reduction in body size. The natural foods which have been given to polychaetes include detritus, cultured phytoplankters (diatoms and flagellates), fresh or dry macroscopic algae, spinach and animals. Liver powder, powdered alfalfa and commercial fish food comprise the artificial foods which have been used.

Polychaete embryos and larvae also can be maintained in the laboratory. The larvae may be procured either by the laborious process of isolating them from plankton and meiobenthic samples or the more ideal method of obtaining early developmental stages by inducing the adults to spawn naturally in the laboratory. Dean and Mazurkiewicz (1975) detail the various culture systems for maintaining larval forms, both pelagic and nonpelagic, as well as appropriate food sources.

d.3 Suggested Arctic Species

Polychaetes used in previous studies, Petinaria hyperborea and Brada villosa (Schneider and Hanes, 1981) and Melaenis loveni (Nortec, 1981), would be logical species to consider. Any local species which is available in sufficient numbers could qualify as a test organism for bioassays.

e) Echinoderms

e.1 Collection and Handling

Many adult echinoderms, especially deep water forms and those which bury themselves, are collected by dredging. In shallower waters, asteroids and echinoids are collected by grab samplers or by diving. For example, the sea urchin Strongylocentrotus drobachiensis has been collected by divers in 7 metre water depths off the western shore of Ragged Island, a site near Cape Hatt, Baffin Island (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). Specimens should be kept cold and damp during transportation.

e.2 Maintenance

Most echinoderms can be kept in aerated aquaria or tanks, often in closed sea water systems. However, if the specimens are to be kept for any length of time an open-water system is virtually the only way to maintain them in healthy condition. Direct sunlight apparently is undersirable or even harmful to most species; dim daylight or dim artificial light, simulating natural diurnal changes, is best. Avoid overcrowding.

Echinoids (sea urchins) are omnivorous opportunists which in the field feed on multicellular algae or on living or dead animals. In the labortaory, they do well on a variety of sea weeds but can also be fed spinach, lettuce, meat from bivalves, annelids, crustaceans and fish, trout food, dog food, frozen shrimp and boiled egg. The food source for asteroids (starfish) depends on their feeding types. Deposit feeders (e.g. Patiria miniata) extrude their stomach and take up food items from the sediment surface. Suspension feeders (genera Henricia, Linckia, Echinaster, Porania) can be maintained on fine particulate matter or phytoplankton. Predatory starfish can be maintained for long periods on small pieces of shellfish as well as the meat of annelids, crustaceans and fish. Many other echinoderms can be kept for several weeks without any food at all if they are kept at cold temperatures.

As part of the Baffin Island Oil Spill (BIOS) Project at Cape Hatt (N.W.T.) the green sea urchin, Strongylocentrotus drobachiensis, was used as one of three test organisms to assess behavioural responses in benthic invertebrates to dispersed crude oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). They were maintained in a flow-through seawater system on site at Cape Hatt in 50 L aquaria at temperature and salinity conditions similar to waters from which they were collected. Subdued natural lighting was used and seaweed (Laminaria sp.) and broken shell material were supplied regularly during the pre-exposure period. The urchins were held in test tanks without sediment at densities that did not exceed field densities.

The larvae and eggs of sea urchins can be used to assay water quality. Gametes of echinoderms are obtained after natural spawning, after gonad dissection or after artifically induced spawning. Kinne (1977) discusses in great detail the rearing of echinoderm larvae as well as environmental and nutritional requirements.

e.3 Suggested Arctic Species

Any local species which can be obtained in sufficient numbers and which withstands the stress of collection and handling is a candidate for use as a test organism. As mentioned in the preceeding section, Strongylocentrotus drobachiensis, has been used as one of three test organisms to assess behavioural responses in benthic invertebrates to dispersed crude oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984) and therefore should be considered as a possible test organism for any future toxicity studies.

f) Crustaceans

f.1 Copepoda

f.1.1 Collection and Handling

Commonly, pelagic copepods are collected using plankton nets. For example, Percy and Mullin (1975) collected the herbivorous copepod, Calanus hyperborea, by means of vertical plankton tows through leads in the Beaufort Sea pack ice. Benthic copepods can be obtained by grab samplers or by divers using an airlift.

f.1.2 Maintenance

Culture methods for benthic and planktonic marine copepods are given in Kinne (1977). Usually natural sea water, filtered and unfiltered, is used as the culture medium, though artificial sea water can also be used. Sometimes antibiotics and chelating agents (EDTA) are added to the medium. Benthic copepods should have the water changed every 3-4 days. Some survive with no food for long periods while others utilize bacteria or a variety of unicellular algae. The culture container for planktonic copepods should be slowly rotated or aerated. There should be frequent water exchange or a recirculation or flow-through system should be used. Excessive handling and too much contact between the copepods and culture-vessel walls should be avoided. Algae such as Dunaliella sp., Isochrysis galbana, Phaeodactylum tricornutum and Skeletonema costatum qualify as good diets for planktonic copepods.

f.1.3 Suggested Arctic Species

The copepod, *Calanus hyperborea*, was used previously (Percy and Mullin, 1975) as a bioassay organism and should be considered in any future studies. Any local species which is available in sufficient numbers could qualify as a test organism.

f.2 Mysidacea, Isopoda, Amphipoda

f.2.1 Collection and Handling

The benthic forms of these crustaceans can be collected using a variety of gear and techniques, including trawls, dredges, grabs, baited traps and divers. The best methods for obtaining intact, healthy specimens are by means of baited traps or divers, equipped with an airlift. The specimens can be transported to the laboratory in plastic bags or containers filled half and half with water and air. Mysids can be collected with plankton nets and transported to the laboratory in buckets of sea water.

Several species of amphipods and isopods have been collected from the western Beaufort Sea for use as bioassay organisms (Percy, 1975; Percy and Mullin, 1975; Percy *et al*., 1978). Benthic amphipods, *Onisimus affinis*, *Corophium clarencense*, *Atylus carinatus* and *Gammarus oceanicus* were readily collected in modified minnow traps lined with fine nylon screen and baited with fish. This method not only yielded adequate numbers of animals in completely undamaged condition but also was the only feasible method of collection through heavy winter ice. *C. clarencense* and *A. carinatus* also were screened from mud samples obtained with a Petersen grab or a light-weight dredge. The amphipods were transported to the laboratory in insulated coolers. The benthic isopods, *Mesidotea sabirica*, *Mesidotea sabini* and *Mesidotea entomon*, usually were obtained by otter trawl. In addition, *M. entomon* and *M. sabirica* were collected in baited traps. *M. sabini* which is a burrowing form was not readily caught in baited traps but was obtained by bottom trawling. These isopods were frequently held 1 or 2 days in large polyethylene tanks of aerated seawater before being transported by air in two gallon jugs of cold sea water (Percy *et al*., 1978).

In a Northern Technical Services study (Nortec, 1981) amphipods (*Onisimus* sp. and *Boekosimus* sp.) from the Prudhoe Bay area of the Beaufort Sea were collected in baited traps which consisted of 18L plastic buckets with three screened hatches. The bait used was either salmon or shrimp. In the same study isopods (Saduria entomon) were collected by hand by divers below the ice and mysids (Mysis sp.) were colleced with plankton nets in shallow waters and transported to the laboratory in 18L buckets of sea water.

Schneider and Hanes (1981) obtained benthic infaunal species (amphipods, Asceroides latipes and Pontoporeia femorata; isopos, Saduria sabini) from near Barrow, Alaska for toxicity tests by using an airlift operated by a SCUBA diver. Epibenthic species (amphipods, Anonyx nugax and Boeckosimus affinis; mysid, Mysis littoralis) were collected with amphipod traps baited with sardines.

f.2.2 Maintenance

These organisms can be maintained in small enclosures which are frequently not aerated, in non-running but regularly renewed water and in larger enclosures (aquaria, fibreglass tanks) in which the water is usually aerated and often recirculated and filtered. Many benthic isopods and amphipods require or prefer the provision of hiding places or substrata such as sand or gravel. Most forms prefer dim light or quasi darkness. Benthic forms have been fed detritus containing bacteria, fungi or protozoans; plants such as diatoms, Ulva lactuca, lettuce, spinach; living animals such as oligochaetes and pieces of molluscs, crustaceans and fish. In addition, various commercial fish foods have been used successfully. Planktonic forms have been fed a variety of phytoplankton, copepods, rotifers, Artemia salina and organic detritus.

As mentioned in the previous section several species of amphipods (Gammarus oceanicus, Onisimus affinis, Corophium clarencerse, Atylus carinatus) and isopods (Mesidotea entomon, Mesidotea sabina, Mesidotea sibirica) have been collected from the western Beaufort Sea (Percy, 1975; Percy and Mullin, 1975; Percy et al., 1978). Sometimes these organisms were held in large tanks of Instant Ocean Seawater at a temperature and salinity that approximated their natural habitat. The water was continuously aerated, filtered and changed regularly. In addition, some of these crustaceans were shipped south to the Arctic Biological Station in Quebec and held in a high capacity refrigerated, recirculating sea water system (Instant Ocean) at a temperature of 1^{o}C and a salinity of 15 - 17 ^{o}/ oo. In both cases the organisms were fed Tetramin fish food. Both these methods apparently maintained these organisms in healthy condition. However, the duration of the maintenance is not stated.

The Northern Technical Services study (Nortec, 1981) utilized a portable laboratory which was located on the sea ice in winter and then relocated on shore prior to spring breakup. The laboratory contained six plywood troughs with flowing sea water and drainage systems. The sea water was pumped into the laboratory through an intake line passing first into large inflatable tanks (air berm) where some settling of suspended material occurred before the water was used for cooling, holding organisms and bioassays. The amphipods (Onisimus sp. and Boeckosimus sp.), isopods (Saduria entomon) and mysids (Mysis sp.) were maintained without food in 50 L tanks set in two cooling troughs in the laboratory. They were held for 48 hours prior to testing with the holding water being changed approximately every 6 hours. This method apparently was successful for maintaining these organisms in healthy conditions. Few mortalities (less than 2 percent) were observed in their handling and holding. However, the length of time these crustaceans were held in the laboratory is not given.

Schneider and Hanes (1981) maintained their bioassays organisms (amphipods, Asceroides latipes, Pontoporeia femorata, Anonyx nugax, Boeckosimus affinis; isopod, Saduria sabini: mysid, Mysis littoralis) at 32 $^{0}/oo$ saliniy, -1.0 °C and in the dark in Percival constant temperature incubators under static water conditions. The holding containers were not aerated but the oxygen content was monitored and did not fall below 75 % of saturation. The organisms were not fed and were usually held for no more than two to three weeks to avoid problems associated with long-term maintenance. Survival was excellent under these maintenance conditions and the animals appeared to be in good health for periods exceeding one month.

f.2.3 Suggested Arctic Species

Any of the species used in the three studies which were cited previously in this section should be considered as bioassay organisms for any future studies. These include the amphipods, Onisimus (= Boeckosimus) affinis, Corophium clarencense, Atylus carinatus, Gammarus oceanicus, Asceroides latioes, Anonyx nugax, and Pontoporeia femorata; the isopods, Mesidotea sabirica, Mesidotea sabini, Mesidotea entomon; and the mysid, Mysis littoralis. In addition, in situ studies (Baffin Island Oil Spill Project) have been carried out to assess the effects of oil and dispersed oil on under-ice amphipod communities (Cross, 1982; Cross and Martin, 1983). The amphipods used in these experiments include the following species: Weyprechtia pinguis, Onisimus litoralis, O. glacialis, Gammarus setosus and Ischyrocerus sp.

f.3 Decapoda Natantia (Shrimp and Prawns)

f.3.1 Collection and Handling

Shrimp and prawns are collected by means of nets, trawls, baited traps or by divers. They can be transported to the laboratory in plastic bags or containers, filled half and half with water and air, preferably in insulated coolers.

f.3.2 Maintenance

Adult shrimp and prawns should be kept in aquaria and related medium-sized enclosures. Decapod larvae can be accommodated in culture dishes (water renewal every second day), compartmented tray or in culture systems in running water. These crustaceans will accept a large variety of food items ranging from plants, invertebrates and fishes to commercial fish foods. However, live foods such as Artemia salina or meat of freshly killed animals (e.g., clams, mussels, squid, crustaceans, fish) seem to be the best diet. The diets consumed by reared decapod larvae include phytoplankton algae such as species of Dunaliella, Chlorella, Isochrysis, Skeletonema, Phaeodactylum, etc., and zooplankters such as rotifers and copepods, as well as eggs and larvae of annelids, cirripedes, molluscs, echinoderms and fishes. Mixed diets are more likely to meet all nutritional requirements. Since benthic natantians such as pink and brown shrimp seek protection in sediments some kind of substratum or protective covering should be made available. Kinne (1977) gives a detailed discussion of rearing both adult and larval decapods in the laboratory.

f.3.3 Suggested Arctic Species

Apparently no Arctic natantians have been used as bioassay organisms. However, any local species which can be collected in sufficient numbers is a possible candidate for use as a test organism.

g) **Bivalve Molluscs**

g.1 Collection and Handling

Adult bivalve molluscs are collected by dredge, grab or diver. The adults of many species experience no adverse effects when transported to the laboratory in containers with cold moist toweling. These organisms can also be transported in containers of cold sea water, preferably in insulated coolers. Bivalve larvae for use in bioassays can be obtained by screening living larvae from plankton samples or by the more desirable method of collecting adults and obtaining gametes from them.

In the Arctic (two sites near Barrow, Alaska) the bivalve mollusc, Liocyma fluctuosa, was collected with an airlift operated by a SCUBA diver for use as a bioassay organism (Schneider and Hanes, 1981). The clams were transferred immediately to buckets of freshly collected seawater to prevent thermal shock. The bivalves, Serripes groenlandicus and Mya truncata, were collected by divers in 7 metre water depths off the western shore of Ragged Island, a site near Cape Hatt, N.W.T. (Engelhardt et al., 1983; Mageau and Engelhardt, 1984) for use as test organisms.

g.2 Maintenance

Juveniles and adults are best maintained in the laboratory in a flowing natural sea water system (closed or open) at controlled temperatures in trays or tanks with several inches of natural substrate (Chanley, 1975; Culliney et al., 1972). The majority of bivalves are suspension feeders and normally the natural food supply in the running water is sufficient to maintain the organisms in good physiological condition. However, supplemental feeding with phytoplankton (natural or cultured) is recommended. The water in an open sea water system can be enriched with suspensions of food algae. In the laboratory bivalves have been raised on a variety of unicellular algae. The deposit feeding bivalves which obtain their food from the bottom detritus will need access to a substratum of sediment.

As mentioned previously the bivalve mollusc, Liocyma fluctuosa, was collected from sites near Barrow, Alaska for use as a bioassay organism (Schneider and Hanes, 1981). These animals were maintained in the dark in Percival constant temperature incubators under static water conditions at -1.0°C and 32 °/oo salinity. Though the holding containers were not aerated the oxygen content of the water was monitored and did not fall below 75% of saturation. Animals were not fed during maintenance but were not held for more than two or three weeks. Survival was excellent under these maintenance conditions.

The two bivalve species (Serripes groenlandicus and Mya truncata) collected from near Cape Hatt, N.W.T. were held in a flow-through seawater system on site at Cape Hatt in 50L aquaria at temperature and salinity conditions similar to those found in the offshore waters where they were collected (Engelhardt et al., 1983;

Mageau and Engelhardt, 1984). The bivalves were maintained under subdued natural lighting conditions and were acclimated for at least seven days. *S. groenlandicus* were kept in a 10 cm layer of washed and sifted coarse beach sand while *Mya truncata* were held without sediment in the tanks. Holding densities did not exceed field densities.

Bivalve larval stages are often used as test organisms for bioassays because they are more sensitive than the adults to environmental conditions. Loosanaff and Davis (1963) and Kinne (1977) contain detailed discussions of the laboratory rearing of bivalve larvae. Larvae can be obtained by screening them from plankton samples but are most often obtained from adults which are collected in the field, conditioned in the laboratory and then stimulated to spawn. Larvae are kept in a variety of enclosures (cylindrical in shape), including Pyrex glass beakers, lobster jars, fibreglass containers, etc. The culture water should be renewed every second day unless running water is used. Use filtered seawater for maintianing the larvae. No food sources other than a variety of unicellular algae such as *Isochrysis galbana*, *Monochrysis lutheri*, *Dicrateria inornata*, *Chlorococcum sp.*, *Cyclotella nana*, etc. have been found satisfactory for maintaining the larvae. Antibiotics, fungicides and germicidal ultraviolet rays are used to treat the sea water for combating microbial growth.

g.3 Suggested Arctic Species

Since the dominant species will vary considerably with location, any local bivalve species which can be collected in sufficient numbers is a possible candidate for use as a bioassay organism. If several sites are being monitored, a common species may not be available and/or abundant at all sites.

Several species of Arctic bivalves have already been used in toxicity studies and should therefore be considered for use in future studies. *Liocyma fluctuosa* collected near Barrow, Alaska, has been used as a bioassay organism (Schneider and Hanes, 1981). As part of the Baffin Island Oil Spill (BIOS) Project two species of bivalves, *Mya truncata* and *Serripes groenlandicus*, were used as test organisms to assess the behavioural and physiological responses of benthic invertebrates to dispersed crude oil (Engelhardt *et al.*, 1983; Mageau and Engelhardt, 1984). In addition, such bivalves as *Portlandia arctica*, *Astarte* sp. and *Macoma* sp. are possible candidates for use in toxicity tests.

h) Fish

h.1 Collection and Handling

The collection of fish is best accomplished by the use of various types of nets or with the assistance of divers. Fish must be handled gently at all times, especially when being transferred. They can be transported in insulated containers of cold freshly collected sea water.

Several species of fish found in the Arctic have been collected for use in bioassays. The fourhorn sculpin, Myoxocephalus quadricornis, has been collected from near the entrance to Eskimo Lakes (western Beaufort Sea) by scooping them up individually in beakers (Percy and Mullin, 1975). In another study (Nortec, 1981) five species of fish (the fourhorn sculpin, Myoxocephalus quadricornis; the broad whitefish, Coregonus nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga) were collected from the Prudhoe Bay area. In the winter a few fish were collected either by hand by divers below the ice or by a fyke net deployed by divers. However, the majority of the fish were collected in the summer and were captured in fyke nets deployed by the divers. Two other species of fish (the Arctic char, Salvelinus alpinus and the least cisco, Coregonus sardinella) were caught frequently but were too large to test in the 50L aquaria used in this study.

h.2 Maintenance

Fish must be acclimated prior to a bioassay, preferably for several weeks. During the acclimation period, they should be actively feeding. Acclimation tanks and accessories must be made of non-toxic materials: glass, stainless steel, or aluminum when organics are important, polyethylene, PVC or polypropylene when metals are important. These tanks should be located away from physical disturbances and preferably separated from test vessels. A constant flow of water through the holding tank is necessary during acclimation. The water should be aerated or oxygenated, cooled and filtered. The photoperiod should probably duplicate the natural one. The tank must be kept clean by periodic siphoning of settled material. To prevent overcrowding there should be at least one litre of water for every 10 grams of fish in the holding tank (Sprague, 1973). A daily inspection for unhealthy fish should be carried out. Water quality (e.g., pH, salinity, temperature and dissolved oxygen) should be monitored. The holding tanks need to be disinfected between batches of fish. Fish should be fed at least twice a day; various types of commercial fish food are available (Kinne, 1977). Bacterial contamination and diseases can be controlled with the use of antibiotics, fungicides and by ultraviolet irradiation of the water. Wong (1982) gives a complete description of the procedures involved in conducting a fish bioassay.

As previously mentioned several species of fish found in the Arctic have been used as biassay organisms. Myoxocephalus quadricornis (fourhorn sculpin) have been maintained in a recirculating seawater system at a temperature and salinity regime approximating that of its natural habitat (Percy and Mullin, 1975). In a second study (Nortec, 1981) a portable laboratory was used which was located on the sea ice in winter and then relocated onshore prior to spring breakup. The laboratory contained six plywood troughs (plywood reinforced with fibreglass) with flowing seawater and drainage systems. Two troughs were used for holding organisms. Seawater was pumped into the laboratory through an intake line passing first into large rubber inflatable tanks (air berm) where some settling of suspended material occurred before the water was used for cooling, holding organisms and bioassays. Water was pumped from the air berm into the laboratory or into two large holding tanks. The fish (the fourhorn sculpin, Myoxocephalus quadricornis, the broad whitefish, Coregonas nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga) were placed in these large holding tanks after collection. They were not fed but some food was introduced with the ambient seawater. Prior to testing, fish were transferred to a holding trough in the laboratory and held for 48 hours without food. Both these methods apparently maintained the fish in healthy condition. However, the duration of maintenance was not given in either study. Pool mortalities in the second study (Nortec, 1981) varied from 2 to 10 percent, fluctuating with alterations in the holding water salintity as weather and currents changed.

h.3 Suggested Arctic Species

Any of the fish species used as bioassay organisms in previous studies should be considered for use in future studies. These include the following species: the fourhorn sculpin, Myoxocephalus quadricornis; the broad whitefish, Coregonus nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga. Rainbow trout have been the standard fish for bioassay work on drilling fluids disposed offshore in the Arctic even though they are not an indigenous species (Wong, 1982). In addition, the ninespine stickleback which is widely distributed in the arctic would seem to be another logical choice for a bioassay organism. However, the threespine stickleback, Gasterosteous aculeatus, apparently is a better choice than either rainbow trout or the nine-spine stickleback for use as the test species in bioassys (Wong, 1982). Additionally, any local species which can be obtained in sufficient numbers and which can survive the stress of collection and maintenance should be considered for use as a bioassay organism.

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APPENDIX A

WORKED EXAMPLE

To help illustrate the planning of benthic environmental sampling and analysis programs in the Arctic, a sampling and analysis plan is developed for a hypothetical situation below. Exact details of logistics, equipment requirements, etc., have not been given.

REQUIREMENT:

Baseline (or background) information is needed in an area that may be subject to extensive offshore exploratory drilling for hydrocarbons from drillships and artificial islands. A contractor is asked to carry out a study at one proposed site. The objectives are given as:

1) characterize the sediments in terms of grain size (percentage sand, clay, silt) and TOC content.
2) determine total concentrations of key trace metals (Hg, Cd, Pb, Cr, Ba) in sediments.
3) determine background levels of petroleum related hydrocarbons (n-alkanes C_{12}-C_{30}, PAH).
4) determine abundance and species richness of benthic communities.
5) determine the body burdens (soft tissue) of trace metals (as above except Ba) and PAH in the most abundant benthic species.

The study is to serve as a basis for future comparisons and an on-going monitoring program: therefore the contractor is asked to employ analytical methods which will be able to quantitatively detect the trace parameters. A quality control program for trace metals and hydrocarbons must be identified and adhered to. The contractor is asked to suggest how many samples should be taken and where.

The contractor is also given the following information:

1) area of influence is expected to be within 2 km of drill site: currents (speed, direction) are unknown. The bottom depths are fairly uniform between 18-22 m within 10 km of the site.
2) Drilling at the site will be from a drillship.
3) No background information exists for the above parameters at the proposed site:
4) the site is ice covered for 9 months on average: the site is outside the zone of landfast ice.

5) closest airfield/harbour/community is 100 km distant.

6) budget for the sampling and analysis is not to exceed $250,000.

7) the study must be completed within 15 months.

A proposed sampling and analysis plan is required for review: the rationale for the study approach and choice of methods is to be given.

DEVELOPMENT OF A PLAN:

1. Assemble Available Information and Assumptions:

a) study area is 2 km radius circle around the proposed site.

b) depth of sediment for chemical analysis - top 1-2 cm as future comparisons will be made to detect recent input.

c) clay/silt particulates should be the focus of analysis as any future contamination will be associated with this size range.

d) centre of drillsite can be regarded as a point source of any waste discharged: concentrations of discharged contaminants likely to decrease exponentially with distance from the source.

e) benthic body burdens and species richness of benthic communities will vary seasonally.

f) no seabottom structures in the area on the basis of hydrographic charts: bottom appears relatively smooth.

2. Sampling Strategy

a) goal is descriptive: however the site will be subject to future contaminant inputs and the survey will serve as the basis for a future monitoring (analytical) studies:

Therefore it is recommended that the study be designed with this in mind: strategy should therefore be to utilize an optimal impact study design (Section 5.3.1) and form and test hypotheses:

b) Study Area: will be a 2 km radius circle centered at the proposed site. A nearby spatial control area will be required which is thought to be outside the expected zone of influence but with similar depth and sediment characteristics.

c) Hypotheses:

H_0 = There is no change in mean abundance (or mean value of percent change in abundance) of the benthic species assemblage in the impact area (over a time interval which includes the impact) relative to the reference area.

H_A = There is a significant change in mean abundance (or mean percent change in abundance) of the benthic species assemblage in the impact area relative to the reference area.

H_0 = There are no changes in environmental conditions (e.g., contaminant body burdens in benthos, sediment grain sizes, TOC, petroleum hydrocarbons, trace metals, depth, salinity, temperature) in the impact area relative to the reference area.

H_A = There are significant changes in environmental conditions (contaminant body burdens in benthos, sediment grain sizes, TOC, petroleum hydrocarbons, trace metals, depth, salinity, TOC) in the impact area relative to the reference area.

d) Where to Sample: nothing is known about potential distribution patterns. Therefore random sampling is best. However, can assume that greatest impact will be close to drill site. Therefore, a useful strategy would be to divide the study area into strata: an inner area of about 300 m in radius: (high impact) and an outer circle: 300 - 2,000 m: (reduced impact) samples within each area would be selected randomly: Sampling effort would be aportioned on the basis of the expected degree of impact (i.e., a more dense allocation of sites in the high impact area).

d.1 Controls: important to incorporate a control area for future comparisons: the choices are:

i) historical (i.e., cores-analyse deeper sections). This is not valid for benthos or hydrocarbons (n-alkanes will be degraded).

ii) third strata beyond 2,000 m: feasible but since actual extent of future effects is not known, effects may extend beyond 2,000 m. Therefore, this is not an optimum configuration.

iii) separate control area: a separate area with a radius of 2 km situated several km away from the study area but in water of the same depth and similar bottom features. This control is the best for future comparisons.

With a reference area as in iii) above and a stratified random sampling, statistical analysis by one-way classification multivariate ANOVA (section 5.3.1.1) would be permitted. This type of design and analysis can be used to test for seasonal changes in the abundance and species richness of benthic populations.

Samples for chemical analysis should be from sediments with a high clay/silt content. If a site is found to be mainly sand or gravel then a new site would be selected.

e) When to Sample: Since benthic community richness and benthic contaminant body burdens will vary with season, a minimum requirement is for winter/summer sampling.

Summer sampling is probably best planned for late August, winter sampling for late March or early April, as these are likely to be the most stable periods in each season in terms of weather and ice conditions and available daylight for on-ice sampling.

f) Number of Samples:

A minimum of 3 sites for each strata required and 3 replicates for both benthos and chemical parameters (Section 3.1.3.2 and 5.). Make an estimate of sampling cost to give an approximate idea of whether two sampling trips are practical within the proposed budget.

f.1 Open water sampling:

Sampling party: 3 (see j.5)
Assume 4 days in field and 3 days travel:

Per diem for sampling party:	$1,000/day x 4 (field) $700/day x 3 (travel)	$ 4,000 2,100
Ship charter: (including fuel, food)	3 days x $4,000	12,000
Return travel to region from south	3 x $1,000	3,000
Equipment leases/rentals (SAT/NAV, etc.)		2,000
Freight		2,500
		$ 25,600
+ 20% contingency in labour and charter		4,000
		✓ $ 30,000

f.2 Through the ice sampling:

Assume 10 field days required.
Assume extra equipment required, helicopter support required.

Per diem:	$1,000 x 7 (field) $700 x 3 (travel)	$ 10,000 2,100
Helicopter	30 hours x $1,000	3,000
Travel (same)		3,000
Equipment lease/rentals		4,000
Freight		4,000
		$ 50,000
+ 20% contingency		10,000
		✓ $ 63,000

Therefore it should be possible to accomodate two sampling periods within the proposed budget.

f.3 For benthos:

Minimum is 3 sites per strata: however, this is a relatively large area so that a larger number of sites is desireable. Therefore suggest 4 randomly selected sampling stations in each stratum as a start, 3 replicate samples at each location.

Therefore 24 samples per area or 48 per sampling period (2 areas).

Total samples would be 96 (48 for winter and summer sampling period).

f.4 For chemical parameters:

f.4.1 Sediment

Unlike biota, chemical content of sediment will probably not vary on a seasonal basis. However, contaminant body burdens will vary: sediments in each survey need to be characterized in terms of grain size and TOC for benthos.

No. of sites as per benthos,
with three replicates per site = 48 samples (one trip only)

plus of the 24 grabs in the control and primary areas, 8 grabs would be selected at random and triplicate subsamples taken from the same grab. Therefore 16 extra samples per area would be collected (32 in total).

Therefore, total number of samples collected (48 + 32) = 80 samples for metals and hydrocarbons.

f.4.2 Biota

It is more difficult to assign a number to tissue analyses since the type of organisms are not known. If the organisms are large enough to provide enough tissue for all analyses then analyses of individual organisms could be considered. However, it will probably be necessary to pool several individual organisms (composite) to obtain sufficient tissue for both PAH and metal analyses.

A suggested sampling design could be:

A minimum of 3 individual organisms to be collected from each of 8 sites in both reference and primary study areas (more if the most abundant organisms are very small). The samples are to be selected from the 3 replicate grabs: the organisms from each grab to be pooled and treated as a single sample.

Therefore number of samples = 32 (16 per sampling period).

g) Samplers

A short armed version of the Van Veen would be used for benthos (Section 5.4.1) since through ice sampling will be required. A Birge-Ekman sampler is a good choice for chemical parameters (Section 3.2) with the Van Veen as a back-up.

g.1 Sample Requirements

Sediment: (per analysis - wet weight)

TOC	5	g
Grain size	100	g
Metals	5	g
Hydrocarbons	50	g
Total	160	g

The cross-sectional area of the Birge-Ekman will be about 500 cm^2. If the top 2 cm is collected and a density of 1.5 g/cm^3 is assumed, about 750 g (wet weight) of sediment should be available. This is more than adequate for all chemical tests.

Tissue: (per analyses - wet weight)

Metals	5	g
PAH	50	g
Total	55	g

h) Sampling Quality Control (Section 3.4)

In addition to the replication indicated, it is recommended that 8 randomly selected individual samples be split into triplicate subsamples for analyses to give a measure of the within sample variability.

i) Logistics:

i.1 On ice logistics (Section 4.3)

i) Transport to and from study area by helicopter (helicopter must be able to take study team plus gear): 2 snowmobiles and sleds to be used to move between sampling sites. These may be driven to the site or carried in a sling under the helicopter.

ii) ice hole: hot water drill: ring diameter to take short-armed version of 0.1 M^2 Van Veen

iii) lift: aluminum tripod and small hand winch for deploying grab and removing ice block.

iv) shelter: portable tent and heater required for on-ice work

v) safety: work on ice; return each night to shore base. However, enough supplies to be taken so that party can stay several days if necessary (food, communications, rifle, sleeping gear).

i.2 Ship-board logistics (Section 4.4)

Charter vessel has hydraulic winch, 3/16" wire rope, booms, davits, accommodation, some work space, large freezer. Need to supply only sampler, storage equipment and benthic processing equipment.

i.3 Freight:

Estimate gear to weigh:

500 kg in summer (750 kg on return)
1200 kg in winter (1500 kg on return).

All equipment to be shipped in labelled wooden boxes. Formalin is restricted item: must be shipped early to avoid delays and packaged separately (Guide 4.2).

i.4 Positioning (Guide 4.3):

A SAT/NAV system is recommended because of price, portability, location and the need for both winter and summer sampling. Accuracy ±100 m is adequate.

i.5 Personnel (Guide 4.5):

A minimum party of 3 would be required for type of sampling gear and sample processing requirements.

i.6 Contingencies:

The winter sampling program may not be feasible as the area is in a region of unstable ice conditions. Therefore a contingency for an extra winter trip may need to be included. Alternatively, the sampling design may need to be revised. The effect of various scenarios should be considered: i.e.,

i) no winter samples possible
ii) not possible to return to summer sites (new sites need to be selected)
iii) only some sites can be sampled
iv) only the primary area or reference area can be sampled
v) reference area needs to be moved

and a course of action decided on if any of the above situations should arise.

3. Analysis

a. Trace Metals

The first decision is that of instrumental technique which depends mainly on the sensitivity required for accurately determining the existing concentrations of these metals in the samples. Assuming that the sediments are typical uncontaminated sediments, the results of section 6.2.3 can be applied.

GF-AAS is chosen for Cd and Pb because F-AAS and ICP are not sensitive enough. The methods of Sturgeon et al (1982) supplemented by the recommendations of Slavin et al. (1983) are chosen. These methods are based on the stabilized temperature platform furnace (STPF), and matrix modifiers are used to enable direct comparison of standards with sample solutions. F-AAS is chosen for Cr and Ba because GF-AAS is too sensitive for these metals, and ICP does not offer a significant cost advantage. Loring and Rantala (1977) and Agemian and Chau (1975a) have provided suitable instrumental methods. CV-AAS is the only instrumental technique that is sensitive enough for determining Hg. The method of MacPherson et al (1982) is chosen. Preconcentration techniques for enhancing the concentrations of Ba, Cd, Cr and Pb in the sediment solutions prior to instrumental quantification are unnecessary.

a.1 Sediment Digestion

A total attack digestion is required, except in the case of mercury where a strong attack method will suffice. As discussed in section 6.2.4.1 total acid attack digestion (with HF) is appropriate for Cd, Cr and Pb, while fusion attack is necessary for Ba. Total attack methods sometimes do not completely dissolve minerals containing barium. The digestion method selected for Cd, Cr and Pb is that of Sturgeon et al (1982). The fusion method selected for Ba is that of Owens and Gladney's (1976) as modified by the recommendations of Mahan and Leyden (1983). (Chromium can also be determined in the fusion attack solutions to check the accuracy of the acid digestion procedure.) For Hg, the strong attack procedure of MacPherson et al. (1982) is chosen. It gave accurate results for Hg in the NRC reference sediments.

MacPherson et al's method can be conveniently integrated into the scheme of Sturgeon et al (1982) since the HCl & HNO_3 digestion of MacPherson et al. is very similar to that employed by Sturgeon et al in the first step of their procedure.

The samples are dried in an oven at 40°C. This temperature should not cause any significant loss of Hg. The dried sediments are disaggregated with a plastic spatula, weighed, and screened with a nylon seive (230 mesh) and the fine fraction is weighed again. This step is done to reduce the influence of grain size effects on trace metal distribution. The fine fraction should then be homogenized and subsampled for the digestions (0.5g for Ba and 1.5g for Cd, Cr, Hg and Pb).

a.2 Tissues

The expected concentration ranges can be less accurately estimated in the case of the biota. Assuming that they are identical to those given in 6.2.4.1, the same conclusions apply.

F-AAS and ICP are sensitive enough for determining Cd, Cr and possibly Pb if preconcentration is employed. Without preconcentration, GF-AAS is sensitive enough for Cd, Cr and possibly Pb. CV-AAS must be used for determining Hg.

Since the organism selected for analysis is likely to be a clam or a worm, it is assumed that the amount of tissue per sample that is available for analysis will be very small. On this basis, the instrument of choice for Cd, Cr and Pb is GF-AAS.

For determining Cd, Cr and Pb, the methods of Hinderberger et al. (1981) supplemented by the recommendations of Slavin et al (1983) are selected. A preconcentration step would be warranted if the sample concentrations are extremely low (or if severe matrix interferences are encountered). The method of Okuno et al. (1978) is selected for preconcentrating Cd, Cr and Pb; concentration factors of up to 100 should be possible with this method. The CV-AAS method selected for determining Hg is Chapman and Dale's (1978). It is a manual method that has excellent sensitivity (L.O.D. = 0.4ug/g). The use of one digestion for all of the metals is desirable, since a significant savings of time and expense may result. The method of Feldman (1974) is selected for the metals, because it is the preferred method for Hg and it compares well with the other methods that are recommended for the Group I metals. A preliminary test procedure is used to determine which variation of the procedure is the most suitable for the kind of tissue sample to be digested. In the case of benthic invertebrates, the SNP variation will probably turn out to be the most suitable, since it is safest for samples containing much lipid. Feldman's (1974) method also has a relatively high throughput, 20 sampler/2.5 hours. This method should be compatible with the instrumental and preconcentration methods already selected for Cd, Cr, Hg and Pb. Sample handling would be a per section 6.2.3. Approximately 300mg (dry weight) per sample is sufficient for Cd, Cr, Pb and Hg.

COST $50 - $100 / sample tissue or sediment

b. Hydrocarbons

Samples from the sampling area would be expected to be pristine with low levels of petroleum hydrocarbons. Thus a major goal would be high sensitivity. For comparison of the hydrocarbon data to data from some other time (i.e. for future monitoring), high accuracy and high precision are required.

The appropriate instrumental method, indicated in tables 7.3.1 and 7.3.2 is gas chromatography with flame ionisation and/or mass spectrometric detection. Sample requirements, indicated in section 7.3.1, are for 50g samples (wet weight) per analysis. Samples should be stored frozen in screw-top glass jars with teflon lined lids, precleaned for hydrocarbons (Section 7.3.2).

b.1 Sediments

Analysis for petroleum hydrocarbons with high sensitivity and high accuracy is described in section 7.3.6 and care in sampling and analysis are emphasized. The objective to analyse for alkanes from nC_{12} to nC_{30} eliminates consideration of the low molecular weight petroleum hydrocarbons.

Sediment extraction, as recommended in section 7.3.6.2, would be by saponification in ethanolic potassium hydroxide and partition into a non-polar solvent. The appropriate fractionation method indicated (Section 7.3.6.2) for this type of analysis is gel permeation chromatography (GPC) followed by silica gel adsorption chromatography.

Analysis of the extracts by high resolution GC/FID and /or high resolution GC/MS will provide the required n-alkane and PAH concentrations and will also allow several related parameters to be calculated (odd-even predominance, pristane/phytane ratio etc., see Table 7.3.3). The utility of such parameters is discussed in volume one (Section III, 3.4.1).

b.2 Tissues

For the PAH analysis, a similar rationale to that used for objective 3) would lead to the analytical goal of high sensitivity, high accuracy and high precision. The appropriate techniques are given in table 7.3.2. This table indicates GC/FID and/or GC/MS be used for in this situation. Sample size and storage reuqirements are indicated in section 7.3.1 and 7.3.2, which recommend 50g wet weight of tissue be collected for each analysis, tightly wrapped in cleaned aluminum foil and stored frozen.

Extraction and analysis of benthic tissue samples is given in section 7.3.1.3 using similar methods that were discussed for objective 3, (petroleum related hydrocarbon). In this case however, only the PAH fractions would be analysed preferably by GC/MS, as indicated in table 7.3.12.

COST $350 - $400 / sample for PAH, n-alkanes in sediments
$250 - $300 for PAH in tissues

c. TOC Measurement: (Guide Section 7.1)

The acid digestion - dry combustion method (Section 7.1.3) is suggested for use because it provides maximum sensitivity for organic material and is more accurate. This is important in a baseline study.

COST $20 - $30 / sample

d. Grain Size

A subsample from each grab of about 100 g wet weight should be taken* -this will be drawn last. Samples should be transferred to a bag or plastic jar. Samples need not be frozen (guide Section 8.2).

Since only clay, silt, sand on a percentage basis is required, and sufficient sample should be available, a combination of a wet sieve and hydrometer sedimentation analysis is suggested. Two sieves would be used: No. 10 (2.0mm) and a No. 230 (0.0625mm). The material passing the No. 230 mesh would be subjected to sedimentation analysis. The material retained on the 230 mesh sieve would be the sand fraction (guide Section 8.1)

COST $60 / sample

* For upper two centimeters assume sediment density wet -- 1.5g/cc: then area of sediment required: $(100/1.5X2)^{1/2}$ 6cm^2

e. Quality Assurance: (guide Section 10; Appendix B, Page 28)

Steps in quality assurance applicable to this study should include:

i) Quality Control

- Analysis of at least one reference material for trace metals in sediment and tissue (guide Section 10.1). Materials selected should have values for all metals if possible: USGS MAG-1 is the only sediment certified for Ba.
- Reference materials should be run with each digestion batch (a minimum of 3).
- Analysis of at least one reference material for hydrocarbons in sediments (frequency as for metals).
- A number of samples (minimum 3) should be homogenized and split and sent to another laboratory for analysis (collaborative testing) — this is especially important for PAH analysis of tissues as no certified reference materials exist.
- 10% of samples will be reanalyzed as blind replicates (i.e. samples are homogenized and split; analyst will not know the identity of the samples).
- Reagent blanks to be run with each digest set (minimum of 3).

- A set of calibration standards (at least 3 different concentrations) to be run with each set of samples for instrumental analysis.

ii) Quality Assessment

- Control charts (Shewhart) to be maintained for
  - reference materials
  - blanks
  - blind replicates

The following criteria would be used in assessing control data (Appendix B, Page B-32):

- Not more than 1 in 20 results be outside 2 standard deviations.

- A point outside 3 standard deviations will require that the problem be identified and corrected before any more samples can be run. Samples analyzed since the last control must be re-run.

- Not more than 7 consecutive results are on the same side of the mean (if so, a systematic error is suggested and the problem identified before more samples are analyzed).

- There are no regular periodic variations.

The cost of the Quality Assurance Program will be about 10-15% of the total trace metal and hydrocarbon costs.

4. **Estimated Cost:** (an estimated cost for the sampling and analysis plan is indicated below)

4.1	Sampling: as per part 2.1	$	93,000
4.2	Analyses:		
	a) Benthos: sorting, processing, identification to species level: average $500 / sample X 96 (range $200 - $800)		48,000
	b) Grain Size: 96 X $60		5,760
	c) TOC: 96 X $30		2,880
	d) Trace Metals in Sediment: 80 samples X $60		4,800
	e) Trace Metals in Tissue: 32 samples X $60		1,920
	f) n-alkanes, PAH in sediment: 80 X $390		31,200
	g) PAH in tissue: 32 X $300		9,600
	h) Quality Assurance (field and laboratory): (add an extra 15% of costs d - g)		7,000
	TOTAL SAMPLING AND ANALYSIS	$	199,160

The cost estimate indicates that all sampling and analyses can be completed within budget: allowing approximately 10% for reporting: (~ $20,000) the total budget would be under $225,000, well within the budgetary constraints.

It would be possible, therefore to suggest sampling additional sites or alternately; to allow for an additional sampling trip in winter if all sampling could not be completed in one trip.

If the estimated cost had been over budget, then cuts would be required. This could be done by reducing the number of analyses. It would be preferable to collect as many samples as possible since it would be very expensive to collect additional samples but relatively inexpensive to add additional analyses if that were required.